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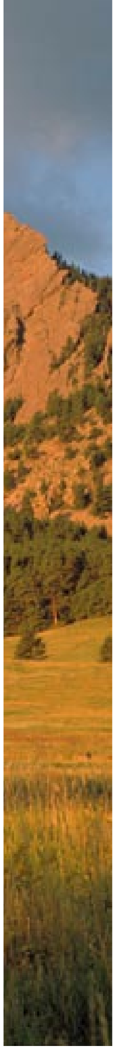

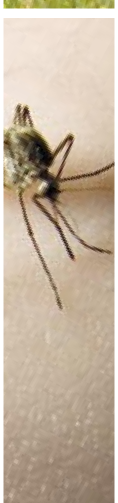

CITY OF BOULDER
WEST NILE VIRUS
MOSQUITO MANAGEMENT PLAN

February 2006

Prepared by:

City of Boulder

Office of Environmental Affairs

In collaboration with

OtterTail Environmental Inc.

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Executive Summary

In an effort to implement a long-term mosquito management strategy to reduce the threat of West Nile Virus (a mosquito-borne virus) to city residents, the city of Boulder has developed this West Nile Virus Mosquito Management Plan. This plan is in compliance with and was guided by the city of Boulder 2002 Integrated Pest Management Policy. In Colorado, two species of mosquitoes are the primary contributors of West Nile Virus (WNV) to humans: Culex pipiens and Culex tarsalis. Beginning in May of each year, a baseline monitoring plan will be implemented and lands owned or managed by the city will be surveyed to evaluate whether either of these two species occurs. Areas that will be routinely surveyed include all known potential mosquito habitat areas found on city-owned lands within the city limits (approximately 524 acres) and on city-owned lands outside of the city limits (approximately 2431acres).

The level of risk from mosquito-borne disease depends on the number of mosquitoes that are capable of transmitting the virus, the prevalence of the virus among them, and the density of the human population in an area where WNV is found. Proper monitoring of larval mosquitoes is an important component in identifying areas that are at high risk for transmission of WNV to humans. Monitoring of larvae can provide information on the expected abundance of adult mosquitoes and can indicate areas where control efforts should be directed. Control efforts during the larval stage are the most cost-effective and efficient means to eliminate mosquitoes at their source and, therefore, to most effectively reduce the risk of human infection. Measures to control mosquito larvae will consist of treatment of breeding habitats with a bacterium (Bacillus thuringiensis subspecies israelensis, or Bti) that attacks the larvae. Control of the larvae limits the possible future need for non-biological control of adult target mosquitoes, such as pesticide fogging or spraying.

Larval Monitoring and Control Plan

Monitoring and treatment of larval sites will be similar to previous years, though the mapped sites have been categorized differently for this plan than in years past. Potential mosquito breeding sites have been categorized based on the combined results of the previous years of larval monitoring. During the years of surveillance, a portion of the potential breeding sites have never been found to be breeding mosquitoes, some sites were found to breeding only nuisance-type (non-Culex) mosquitoes, and other sites were found to be breeding Culex mosquitoes and received one or more control treatments. Therefore, it makes sense to differentiate between habitats and focus initial efforts at sites where Culex almost certainly will occur and/or where Culex mosquitoes are more likely to occur at some point during the season. Based on data collected from 2003-2005, the sites have been categorized as follows:

Category I - Vector larval breeding sites: All sites where Culex larvae have been found breeding

Category II - All mosquito larval breeding sites: All sites where only nuisance mosquito larvae have been found breeding (to date)

Category III - All potential larval breeding sites: All potential mosquito breeding sites that have not been found breeding any type of mosquitoes (to date)

In general, Boulder's mosquito season occurs May through September, while the peak season for Culex breeding is June through August. Larval monitoring and control will occur at all Category I sites once per week, May-September. Category II sites will be monitored once per week during the height of the Culex breeding period, June-August. Monitoring at Category III sites (all remaining mapped potential larval breeding sites) will occur once during the peak season as a measure of quality control. Since climate can be a large contributor to seasonal Culex habitat trends, seasonal temperature and precipitation patterns will also help determine the frequency at which sites will be monitored.

The plan also allows for maximum flexibility in monitoring and control, should a combination of thresholds be met at any point in the season that may warrant increased vigilance and/or management. Thresholds for increased larval monitoring between site categories include:

- A significant percentage (~50%) of Category I sites that are breeding Culex larvae in May,
- Early detections of WNV in area adult sentinel traps,
- High or rapidly increasing adult Culex mosquito populations in general, and
- Early or rapidly increasing cases of WNV in humans and/or birds.

Adult Monitoring and Contingency Control Plan

Proper monitoring of adult mosquitoes, which includes testing for the presence of WNV, is important in guiding prevention and control because it can provide information on the potential threat to residents and can indicate areas where efforts to eliminate mosquitoes should be targeted. Adult mosquito monitoring will continue as in years before in the established trap locations. All 16 city traps are monitored weekly for WNV in adult mosquito populations during the peak of Culex activity (June-August). The city's adult monitoring does not cover the entire length of mosquito season (or potential Culex breeding period), though forewarning of especially early or late season Culex activity in adult traps can be gathered from contacting partner agencies on a regular basis.

Monitoring adult traps for WNV presence will occur earlier (May) or later (September) or more frequently than planned if data from local partner agencies (Colorado Dept. of Health, Boulder County Public Health, Colorado University) indicate that there are early, rapidly increasing, or high sustained levels of Culex mosquito populations and/or early, elevated, or sustained cases of WNV present in birds and/or humans. A continued and extensive communication network will serve as the best resource to make the most informed decisions on monitoring and control of WNV. This network involves city agencies, regulatory agencies, and other bodies associated with monitoring of WNV.

The presence of mosquito-borne pathogens in Boulder, if detected, will prompt one or more responses or interventions. Monitoring data will be used to assess the risk of an outbreak of human disease and the need to apply pesticides in a limited and targeted area to control adult mosquitoes. The control response will depend on a combination of thresholds being met that include, but are not limited to:

- the overall intensity and persistence of the WNV activity in adult Culex mosquitoes, humans, birds, and non-avian vertebrates
- the proximity of WNV activity to human populations within the city,
- the time of year,
- vector index level, and
- seasonal climate.

Final recommendations on adult control activities will weigh heavily on recommendations from the City of Boulder management staff, partner agencies, Center for Disease Control (CDC), and Environmental Protection Agency (EPA). The thresholds established for implementation of adult mosquito control activities is based on finding WNV in C. tarsalis or C. pipiens adult females. Meeting or exceeding these thresholds does not directly correlate to spraying pesticides every time WNV is discovered in adult mosquitoes. Control for adult mosquitoes will be determined on a site-specific basis.

Background

West Nile Virus is a disease that was first detected in the United States during the summer of 1999 in New York City and has since spread westward across the U.S. During the 2003 mosquito season, Colorado led the nation in WNV human cases (2,945) and WNV-related deaths (55) (CDPHE 2004). WNV activity and human cases have since decreased in Colorado, but continues to be an annual threat. WNV is maintained in the bird population. Mosquitoes are usually not carriers of WNV until they bite an infected bird. An infected mosquito may then pass the virus on to humans, horses and other animals through an additional bite. Although many people who contract WNV experience no or very mild symptoms, WNV infection can result in severe and sometimes fatal illnesses.

There are approximately 54 species of mosquitoes in Colorado, yet only the species from the genus Culex are known to be effective transmitters of the virus, also known as vectors. The city of Boulder's control plan focuses only on controlling vector populations since other mosquito species are not directly involved in the disease transmission process. Through surveillance of potential mosquito breeding sites, areas identified as containing vector species are identified and treated with biological control agents. This method of targeting prevents the mosquitoes from developing into adults and transmitting the virus, and is the most cost effective and accurate way to reduce vector populations. In addition, control of the larvae limits the possible future need for non-biological control of adult mosquitoes such as ultra-low volume (ULV) pesticide spraying.

Prior to the WNV outbreak in Colorado in 2003, the city of Boulder did not historically control mosquitoes. The city's policy was that the nuisance created by mosquitoes was not significant enough to warrant pesticide application. Although the city had not been controlling mosquitoes on its lands, Boulder County had been controlling mosquitoes on unincorporated county lands and various other lands not owned by the City. These control efforts are administered by the Boulder County Public Health department (BCPH) and are designated as the Boulder County Mosquito Control District. The Boulder County Mosquito Control District was delineated by the county commissioners and is funded by a tax on the residents of the district. The county's control program has historically focused on controlling nuisance mosquitoes and activities including control of both larval and adult mosquitoes.

The program for controlling mosquitoes described in this plan differs from the county's past efforts in two primary ways. First, the city is currently responsible for funding its own mosquito control efforts. Second, to maximize the protection of the environment, the city is not attempting to control nuisance mosquitoes, but instead, is focusing its efforts on protecting human health by controlling only the mosquito species that can effectively transmit WNV. Therefore, any mosquito breeding habitats that do not contain the species targeted in this plan will not be treated, even if significant numbers of nuisance mosquito species are found. Despite the differences between the goals of the city's program and the county's program, both parties are working closely together to assure that a collaborative effort is undertaken to minimize the risk to human health from WNV.

History of City of Boulder WNV Program

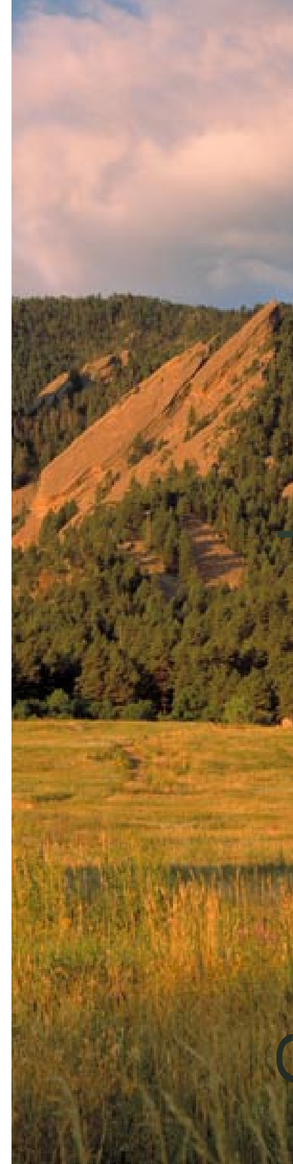
WNV first appeared in Colorado in humans in 2002. Trends in the spread of the virus predicted that the spread of WNV would continue westward and that cases in Colorado would likely be much higher the following year. As predicted, Colorado led the country in WNV cases in 2003. In a proactive measure, the city of Boulder contracted with OtterTail Environmental, Inc. in early 2003 to develop and implement a mosquito control program that both reduced the risk of WNV to human health and minimized adverse effects to environmental health and function. The program focus is on prevention and early detection and control of vector larvae in the Culex genus.

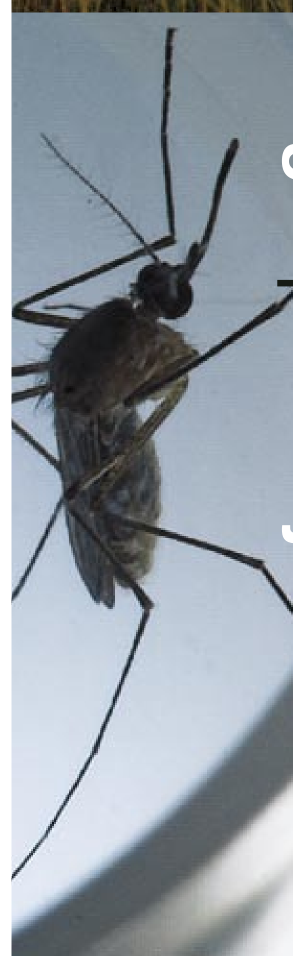
As part of the early detection work, OtterTail surveyed and mapped all potential mosquito breeding habitat on city-owned properties within or near city limits in 2003 and 2004. This map (see Figure 2-1), which serves as a basis for all monitoring efforts, identifies 488 individual potential breeding sites that cover 2,955 acres.

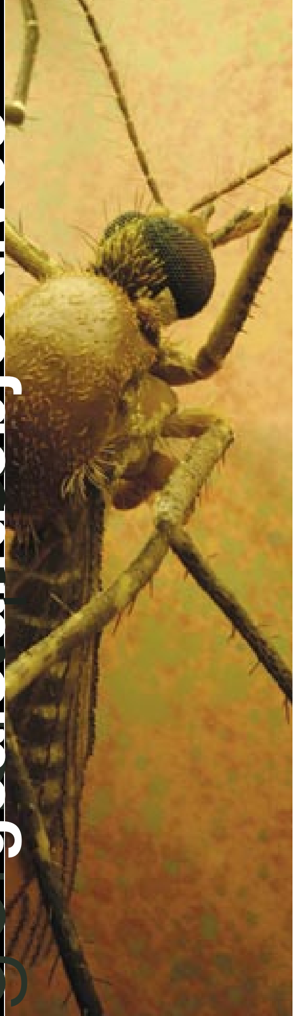
Since 2003, the program has become more refined. As a result of three years of monitoring data, the program is now able to implement a more cost-effective monitoring and control approach that continues to protect human and environmental health. The development of control thresholds and categorized sites based on vector presence has allowed the city to identify an annual baseline monitoring system (presented in this plan) for mosquito control and WNV prevention that is more efficient, reliable, and cost-effective.

Goals and Objectives

The overall goal of this WNV control effort is to reduce the risk of human WNV infection while providing the most limited effect to the environment possible. This goal will be achieved through a long-term mosquito monitoring and control program that is responsive to the public, environmentally sound, and cost-efficient. This plan is directed primarily at controlling the larval stages of mosquitoes and their sources. Control will generally be contained in localized areas to lessen the environmental impacts. Control of adult mosquitoes may be employed, but only in emergencies, after certain criteria are met which warrant adult treatment. The comprehensive nature of this plan ensures the efficacy of the control measures while minimizing adverse impacts to human health and the environment.







The objectives that have been used to accomplish this goal are:

Objective 1: Categorize the habitats that support mosquitoes that most effectively transmit WNV to humans. This categorization allows the city to focus its initial monitoring and treatment efforts in habitats that have bred Culex species or could potentially breed Culex larvae, and, therefore, will reduce the number of treated sites compared to standard control practices (many other control programs across the country treat all habitats where significant mosquito populations are found, regardless of the species). Targeting efforts in this way will substantially reduce the environmental impacts to the city's wetlands and other water resources as well as labor costs of automatically surveying every wet area, regardless of Culex suitability.

Objective 2: Apply the larvicide (Bti) to all sites where Culex species are found. This effort reduces the potential for adult vector mosquitoes to hatch. It also lowers the potential need for spraying with pesticides that are more hazardous if WNV becomes an epidemic.

Objective 3: Use adult mosquito monitoring to provide an early warning system of the occurrence of WNV within and near city limits. Data collection will include: (a) conducting direct WNV testing in the adult mosquito populations of the two species of mosquito that most effectively transmit WNV to humans; and, (b) coordinating with local and state agencies on the results of this testing. Data will also be collected on the locations of actual WNV outbreaks (for mosquitoes, birds, and mammals) on lands owned by the city.

Objective 4: Develop trigger mechanisms to respond to early larval detection and/or heightened mosquito activity to appropriately increase management activity.

Objective 5: Utilize thresholds for initiating adult mosquito control in emergency cases.

Objective 6: Continue the program to educate the public about WNV and increase awareness of the city's WNV Mosquito Management Plan. This program will inform the public about the need for efforts to control vector mosquitoes and will describe what these efforts will entail.

CHAPTER 1: NUISANCE MOSQUITOES

Mosquitoes pose both a nuisance problem and a West Nile Virus threat to a community. There are over 50 species of mosquitoes in Colorado -two of them are known to effectively transmit West Nile Virus to humans. Nuisance mosquitoes are those mosquitoes that do not carry disease. For example, "flood water" mosquito populations can increase after significant rainfall amounts and are aggressive biters, but they are not known to transmit WNV to humans. Nuisance mosquito population trends have stayed fairly constant in the Boulder area over the past several years. However, because of West Nile Virus, the public seems to be more in-tune and concerned about nuisance mosquitoes than in the past.

In this plan, the city of Boulder has a comprehensive and proactive approach to managing mosquitoes that may carry WNV. The plan focuses on larval control of the two species that can most effectively transmit WNV, Culex tarsalis and Culex pipiens. All habitat sites with Culex occurrences are treated with Bti (Bacillus thuringiensis israelensis), since larval control is the most effective way to reduce mosquito populations. This plan also includes emergency spraying/fogging of adult mosquitoes if thresholds for spraying are met. Sixteen adult mosquito traps are placed throughout the city and are monitored weekly from June through August to determine the number of Culex mosquitoes present in the area. Community outreach and education is essential in helping individuals understand WNV and take simple precautions that can be taken to help prevent a disease outbreak. The city's strategy is a balanced approach of individual responsibility and city action to control, as reasonably as possible, the threat of WNV.

The city's Integrated Pest Management (IPM) policy (Appendix A) provides direction for managing pest and nuisance problems including weeds, insects, and animals on public lands. This policy directs that the least toxic controls be used and that "alternative" methods be taken before spraying pesticides is considered. Spraying pesticides is only done as a last resort.

Non-chemical methods and means to be considered to control nuisance mosquitoes follow the IPM protocol of (1) prevention/ education (2) cultural controls (3) mechanical controls and (4) biological controls. The following are some examples of these types of controls:

Prevention/Education:

- Information for the prevention of larval development and adult mosquito bites
- Limited public distribution of repellant at some community facilities

Cultural Control
- Removing mosquito harborage areas where possible

Mechanical Controls
- Mosquito magnets
- Street sweeping to help move standing water in curb lines
- Storm drain cleaning of debris so water moves

Biological Controls
- Bat and bird houses - encourage natural mosquito predators

CHAPTER 2: LARVAL MOSQUITO MANAGEMENT

Efforts to control mosquitoes conducted during the larval stage of mosquito development are the most effective and environmentally sound way to eliminate target mosquitoes and, therefore, to most effectively reduce the risk of human WNV infection. The U.S. Environmental Protection Agency's (EPA) Office of Pesticide Programs is responsible for ensuring that a pesticide will not pose unreasonable adverse effects to human health and the environment. To prevent and minimize the impacts of pesticides on fish, wildlife and plants, the U.S. Fish and Wildlife Service provides technical assistance and consults with the EPA during registration and re-registration of pesticides. In Colorado, pesticide rules and regulations are administered and enforced by the Colorado Department of Agriculture, Division of Plant Industry.

Preferred Larval Control Agents

Larval control agents (larvicides) are materials that target the larval stage of the insect and prevent mosquitoes from becoming adults. The preferred larval control agent proposed for use under this plan is the microbial insecticide Bacillus thuringiensis subspecies israelensis, or Bti. This product is discussed below.

Application of Bti

Bti is a microbial insecticide formulated for use to control mosquito larvae in aquatic habitats. The product is manufactured as corncob granules and is applied by hand or by using hand-held seeders (spreaders) and power spreaders. Bti is an augmentative biological control agent formed from bacterium that occurs naturally in soils. The bacterium produces protein crystal protoxins during the formation of spores that disrupt bodily functions in some insects (Extension Toxicology Network [ETN] 1996). This active ingredient of Bti is called a crystalline delta-endotoxin. Live bacteria are not contained in Bti; the active ingredient is separated from the bacteria that are killed in a laboratory. When they are ingested by the mosquito larvae, the protoxins dissolve in the intestine and the delta-endotoxin reacts with the stomach secretions. The cells in the gut then become paralyzed, interfering with normal digestion and triggering the insect to stop feeding (ETN 2003). Death typically occurs within a few hours of ingestion.

Bti adversely affects larval stages of species in the Order Diptera, Suborder Nematocera, Family Culicidae (mosquitoes). Research and field experiments have shown that Bti has no toxic effects on beneficial and predacious arthropods or insects such as honeybees, beetles, mayflies, dragonflies, damselflies, stoneflies, caddisflies and true bugs (CMC 2003). CMC (2003) also found that among Diptera (true flies and midges) Chaoborus species, Ephydra riparia, Musca domestica, Odontomyia species, and Polypedilum species demonstrated no susceptibility to Bti. It was also determined that variable mortality did occur among Chrironomus pulmosus, Chrionomus stigmaterus, Dixa species, Goeldchironomus holoprasinus and Palpomyia species (CMC 2003). Low levels of toxicity were also observed among a few species of butterflies and moths, but CMC (2003) found no toxic effects occurring in crustaceans or amphibians.

Using Bti to control larval mosquitoes offers several advantages. First, its residual lasts only 24 hours in water, and it breaks down rapidly as a result of exposure to ultraviolet light (EPA 1998, 2002; ETN 2003; National Pesticide Telecommunications Network [NPTN] 2000). Second, it does not affect nontarget vertebrate species, such as fish and birds (EPA 1998 2002; ETN 2003; NPTN 2000). Third, the bacterium kills the mosquito larvae and field technicians can observe the results the same day the Bti is applied. A negative effect is that part of the food chain is temporarily removed by killing the larvae and other dipterans, potentially affecting predators by removing a source of food. However, because Bti does not last long in water, adult mosquitoes and other dipterans could lay eggs in the treated water 24 hours after a treatment, and larvae could develop to provide another source of food to predators. Treatments are usually made after the larvae have been available to predators for up to two days of the normal four to five day larval stage.

The application of Bti has been determined in this plan as the preferred method for larval mosquito control because it serves the greatest public need. The threat that WNV will infect residents of Boulder outweighs the impacts of this bacterium on the areas where it would be used. Larval treatments must occur to help contain and minimize the threat of WNV infection in humans. Adverse impacts to areas being treated will be minimized by applying Bti at recommended concentrations, and all treatment areas will be posted with signs before Bti is applied.

LITERATURE REVIEW OF TOXIC EFFECTS FROM *BACILLUS THURINGIENSIS* SUBSPECIES *ISRAELENSIS* ON NON-TARGET ORGANISMS

Non-target Organism	Effects	Comments[1]
Small laboratory mammals (rat, rabbit)	No unacceptable acute toxic effects	Based on standard acute testing via inhalation, dermal exposure, and eye irritation
Human infants & children	No toxicity	Prediction included 10x margin of safety and special exposure patterns.
Humans	No endocrine or immune system effects	No evidence known to exist by the EPA.
Birds (mallard, bobwhite quail)	No toxicity	Based on standard acute and subacute testing.
Insects (green lace-wing larvae, parasitic wasps, predaceous water beetles)	Little to no toxicity	Green lace-wings—16 day $LC_{50} > 1.5 \times 10^8$ colony-forming units (cfu)/g diet; 16 day no-observed-effects-level (NOEL) = 2.5×10^4 cfu/g diet. Wasps—30 day $LC_{50} > 7.9 \times 10^7$ cfu/g diet. Water beetles—9 day $LC_{50} > 1.8 \times 10^8$ cfu/g diet.
Honey bee	Minimal toxicity	5 day $LC_{50} > 7.0 \times 10^7$ cfu/g diet.
Fish (trout, bluegill)	No toxicity	Trout—aqueous $LC_{50} > 8.7 \times 10^9$ cfu/l and oral $LC_{50} > 1.7 \times 10^{10}$ cfu/g food, slightly toxic; aqueous $LC_{50} > 1.4 \times 10^{10}$ cfu/l and oral $LC_{50} > 5.3 \times 10^9$ cfu/g food. Bluegill— aqueous $LC_{50} > 8.9 \times 10^9$ cfu/l water and oral $LC_{50} > 1.3 \times 10^{10}$ cfu/g food; aqueous $LC_{50} > 1.6 \times 10^{10}$ cfu/l and oral $LC_{50} > 4.3 \times 10^9$ cfu/g food.
Freshwater invertebrates (Daphnia)	Moderately toxic	21 day LC_{50} = 5-50 ppm.
Plants (terrestrial, semi-aquatic, aquatic)	Minimal risk of toxicity	No evidence known to exist by the EPA.
Mammalian wildlife	Minimal to nonexistent risk of toxicity	Based on known mammalian studies and 30 years of use.
Soil invertebrates (nematodes, ground beetles)	Toxic (all strains of *Bti*)	Toxic to some nematode and ground beetle species; indirect adverse effects likely for predators of such species.
Invertebrate predators (flies, dobsonflies, dragonflies, stoneflies, caddisflies)	No negative impacts	Study (Merritt and Wipfli 1994) included field and lab testing for three years.[3]
Non-target invertebrates (nearly 100 species)	No ill effects	Reported by Garcia et al. 1980.[4]
Non-target aquatic insects (caddisflies, mayflies, stoneflies, damselflies, dragonflies, water beetles), aquatic invertebrates (Daphnia, rotifers, crustaceans)	No demonstrated effects	
Aquatic vertebrates (trout, sunfish, frogs, salamanders), terrestrial vertebrates (mallard, mammals)	No negative effects	
Benthic macroinvertebrate communities (179 genera of aquatic insects from 7 orders) in 27 wetlands	Delayed toxicity to many dipteran genera, mostly chironomids, and some non-dipteran predator genera	Total insect density significantly reduced by 60 percent in second and third year of normal treatment. Total dipteran density significantly reduced by 62 percent and 82 percent in second and third year of normal treatment. Total insect richness (number of genera) significantly reduced by 33 percent to 67 percent in second and third year of normal treatment. Chironomid richness reduced by 43 percent and 66 percent in second and third year of normal treatment. Non-dipteran predator genera richness reduced by 64 percent in third year of normal treatment. Results indicate a profound change in wetland function and indirect adverse effects are likely for vertebrate predators of chironomids (such as waterfowl).

Note: [1] LC50 = Lethal concentration to 50 percent of test population. Sources: EPA 1998, Joung and Côté 2000, Merritt and Wipfli 1994, Garcia et al. 1980, Agriculture Canada 1982, and Hershey et al. 1998.

Table 2-1 Literature Review of Toxic Effects from Bti on Non-Target Organisms

The Material Safety Data Sheet (MSDS) for Bti is included in Appendix B. Proper handling of Bti and procedures for spill control are summarized later in this chapter. Application requirements proposed for use under this plan are discussed in later sections.

Potential Impacts of Bti to Nontarget Species

The bacterium Bti has been studied and used as a mosquito larvicide for about 20 years. Hundreds of peer-reviewed scientific journal articles and government agency documents have been published about the potential for ecological effects from Bti. A review of several summary publications from reputable federal agencies suggests that Bti is non-toxic to nearly all types of nontarget organisms (in other words, the target organisms for Bti are all mosquitoes and black flies), including other insects, with two exceptions. Bti is toxic to microorganisms in soil (such as nematodes) and may be toxic to Daphnia (a genus of freshwater invertebrate crustacean), according to the EPA and Agriculture and Agri-Food Canada. This information is summarized in Table 2-1.

Most of the studies cited by the summary documents were conducted in the laboratory for a short duration and considered only the effects to the study organisms themselves. Two studies published in 1998 provide contrasting evidence of more wide-ranging effects to nontarget species. FCCMC (1998) concluded that, although Bti is highly effective in killing the targeted mosquito larvae (C. tarsalis and C. pipiens), it is also lethal to many other insect species in several taxonomic orders besides Diptera (flies). Some larval mortality of insect species that are normally associated with mosquito larvae in aquatic habitats was observed in the Families Chironomidae (midges), Ceratopogonidae (biting midges), and Dixidae (dixid midges). However, the concentration of Bti required to cause this effect was 10 to 1,000 times higher than normal application rates for controlling mosquitoes.

Hershey et al. (1998) evaluated Bti applied for 3 years at normal rates to 27 wetlands and determined adverse impacts to insect and non-insect orders were delayed until the second year of application. In the second and third years of application, significant reductions in insect abundance and insect species richness were observed. Overall, insect community composition was radically altered, and the authors speculated that many species were adversely affected indirectly via changes to the ecosystem structure. The authors also estimated that wetland function had been disrupted and degraded, likely causing indirect effects to wetland vertebrates that could not be measured in the 3-year time frame.

Monitoring Study of Local Bti Impacts

An assessment of the affect of Bti on nontarget aquatic benthic organisms was conducted by Ottertail Environmental at a predetermined site in Boulder in August of 2003, 2004, and 2005. Quantitative Ponar samples were taken of the mosquito breeding habitat bottom substrate before, and three days after, the application of Bti. A control site was also sampled in a similar habitat type without the application of Bti.

The aquatic substrates in the study area were highly heterogeneous, making it impossible to obtain replicate samples that captured the same microhabitat. This, combined with the knowledge that lentic (pond) habitats are typically difficult to obtain reproducible results, continued to make reliable trend analyses of this data set difficult at best. When comparing the total abundance numbers throughout the study period, there is a noticeable decrease observed from 2003 to 2005. However, the control had a similar decrease, suggesting that Bti was not the reason for the decrease over time.

Many studies at a research-level analysis have been conducted over the years to determine the effects of Bti to nontarget organisms. These studies would be a more reliable tool for interpretation of probable impacts to nontarget organisms within the city of Boulder's project area. A compilation of the results of these studies are presented in the literature review (Table 4-1). For these reasons, the local assessment study of Bti on nontarget aquatic benthic organisms was discontinued in 2006.

Potential Effects of Bti to Human Health

Bti poses little threat to human health through either handling the product directly, or being exposed to it indirectly, such as during a municipal mosquito control program. Bti is essentially non-toxic to humans, so there is minimal concern for human health effects from Bti when used according to labeled directions (EPA 2002). The most likely routes of exposure for the general public to Bti are oral, dermal, and inhalation. Slight to moderate skin irritation and eye irritation has occasionally been observed in product tests, which may be attributed to other ingredients in the product formulation (EPA 1998). Observed eye irritation is often associated with dry, anhydrous forms of the product and may be due to physical irritation effects as might be caused by sand or drying agents rather than toxicity of the product. Product labeling requirements regarding these effects are in place to minimize the risk of skin or eye irritations.

The acidic conditions in human stomachs do not activate Bti toxins. Studies have shown that even if Bti spores are ingested or inhaled by humans, they are eliminated without any adverse health effects (Health Canada 2001). To date, no known mammalian health effects have been demonstrated in any infectivity / pathogenicity study of Bt-based products (EPA 1998).

Potential Impacts to Water Quality from Bti

Bti is not expected to impact public drinking water supplies. Bti is a naturally-occurring soil bacterium that readily breaks down in the environment through exposure to sunlight and microorganisms. The potential is minimal for Bti to enter groundwater or other sources of drinking water, and the bacterium does not proliferate in aquatic habitats (EPA 1998). Bti is not screened for in drinking water supplies as a potential indicator of microbial contamination or as a direct pathogenic contaminant (EPA 1998). Bti is suitable for application to irrigation water and any other water supplies, except for finished drinking water. Low percolation rates through soil and municipal treatment of raw water supplies used for drinking water reduce the possibility of exposure to Bti through drinking water sources. As a safety precaution, labeling requirements must state that Bti should not be applied directly to treated drinking water reservoirs or drinking water receptacles. Bti does not readily move from an application site and is unlikely to percolate to groundwater (National Pesticide Telecommunications Network [NPTN] 2000). No restrictions have been issued for use of this material around bodies of water (EPA 1998).

Potential Effects to Water Quality from the Manufacturing Process of Bti

Bti is grown commercially in large fermentation vats under a controlled environment. The material is then concentrated, dried, and formulated for use as a liquid or a solid. In addition to the active ingredient Bti, other ingredients are used to make the final product. These other ingredients are termed “inert” ingredients, as they do not have direct pesticidal activity against the target pest. Product manufacturers consider the inert ingredients proprietary information. Unless an inert ingredient is determined to be highly toxic, it is not required by law to be identified by name or percentage on the product label, however, the total percentage of inert ingredients must be declared (NPTN 2000b). No toxic materials or hazardous chemicals (that is, heavy metals) are used in the manufacturing process of Bti (Whitman 2003). Some strains of Bt, of which Bti is a variety, have the potential to produce various undesirable toxins during the manufacturing process, however, quality control testing requirements are in place to prevent these toxins from appearing in products (EPA 1998). The disposal of any residual material from the manufacturing of Bti is not expected to have any environmental impacts due to the non-toxic nature of the ingredients used in the process. Effluent from the Bti manufacturing plant may contain Bti or fermentation byproducts; discharges of this effluent to waters of the state would be regulated under the National Pollutant Discharge Elimination System permit process for discharges from the plant to ensure compliance with state water quality standards.

Managing Flood-Irrigated Lands on Open Space and Mountain Parks lands

Flood-irrigated lands, identified by the city of Boulder, were mapped and surveyed for the presence of mosquito breeding habitats. Operators on these lands will be contacted to discuss their plans for irrigation and irrigation frequencies. Irrigators will be encouraged to reduce the water volumes applied during flood irrigation to maximize agronomic uptake and minimize the amount of standing water on these lands following irrigation events. Mosquito breeding habitat would be minimized by reducing the amount of standing water. These areas will be surveyed throughout the mosquito breeding season and larval treatments would be applied when the targeted Culex species are identified.

Other Larval Mosquito Control Methods

Two additional larval control techniques were considered but are not preferred at the current time. However, theses techniques were not rejected as options for future management should ecological and/or risks to human health conditions change. They include application of the insecticide Bacillus sphaericus and manipulation of mosquito habitats. These methods are discussed below.

Application of Bacillus sphaericus

Bacillus sphaericus (Bs) is a bacterium that occurs naturally in soil and contains protein crystals and living spores with larvicidal abilities similar to Bti. The toxin is active only against the feeding larval stages and must be partially digested before it becomes activated. During digestion, larval enzymes dissolve the crystals in to protoxins, which are smaller crystals. These protoxins then paralyze the gut and break through pores in the gut wall within a few hours to invade the body cavity and multiply. The mosquito larvae will die within 48 to 72 hours allowing predators a minimum of 2 days of the normal 4 to 5 day predation window.

Bs adversely affects larval stages of insect species in the Order Diptera, Suborder Nematocera, Family Culicidae (mosquitoes) (Florida Coordinating Council on Mosquito Control [FCCMC] 1998). Bs is specific in causing mortality to mosquito larvae. Culex species are the most sensitive to Bs. In contrast to Bti, Bs is virtually non-toxic to black flies (FCCMC 1998). Mammals and other non-target species are unaffected by applications of Bs.

The application of Bs is an option for control of WNV, but is not proposed for use under this plan. It is similar to Bti in that it is a bacterium, but the differences are significant. Bs is desirable because, like Bti, it kills the mosquito larvae, and field technicians can see results within two days of treatment. Bs also has demonstrated efficacy in controlling mosquito larvae in highly organic aquatic environments, including sewage-waste lagoons and septic ditches (FCCMC 1998).

However, certain factors make this product undesirable for use in the city of Boulder. First, the residual for Bs in water is 2 to 4 weeks before retreatment is necessary (FCCMC 1998). Bs has the ability to release fresh spores into the water column and recycle itself offering residual control, but also having extended affects to nontarget organisms. Second, mosquitoes have been shown to develop resistance to Bs, which reduces its effectiveness (Nielsen-LeRoux and Silva-Filha 2003). Finally, similar to Bti, part of the food chain is temporarily removed, potentially affecting predators by removing a source of food. Eggs that are laid within 4 weeks of treatment still have the potential to be affected by Bs, causing a break in the food chain that lasts longer than with Bti.

Mosquito Habitat Manipulation

Manipulation of mosquito habitats is another option for control of WNV. Manipulation involves water management strategies to eliminate mosquito breeding areas and can include activities such as filling in or improving drainage in certain areas, or pumping water out of low-lying areas. Manipulation can permanently change the function of the mosquito habitat and can severely affect the ecological integrity of the wetland ecosystem. This method of mosquito larvae control would be implemented only on nonregulated wetlands under this plan.

Techniques Considered But Not Recommended for Larval Mosquito Control

Three optional techniques for controlling larval mosquitoes were considered for use in this plan but are not recommended. These options include applying the insecticide methoprene, introducing predatory fish, and applying oils or monomolecular surface films. These methods are discussed below.

Application of Methoprene

Methoprene is different from Bti and Bs because it is a hormonal insect growth regulator (IGR), not a bacterium, and it does not immediately kill the mosquito larvae (EPA 2002). The IGR is a copy of the juvenile hormone in the mosquito. The hormone prevents complete metamorphosis by disrupting the molting process and does not allow the larvae to develop into an adult causing the mosquito to die at the pupa stage (EPA 2002). Methoprene allows the larvae to remain in the food chain, but prevents the emergence of adult mosquitoes that bite and breed. The methoprene is added to the water and absorbed through the larval exoskeleton.

Use of methoprene in wetlands poses two identified maximum potential impacts. First, it affects more nontarget species than Bti and Bs, including fish and aquatic invertebrates. Second, the residual time for methoprene in water varies depending on the form of the product used: 21 days (sand), 30 days (pellet), or 150 days (briquette) (MMCD 2002). This long residual time poses a significant risk to the biological function of wetlands.

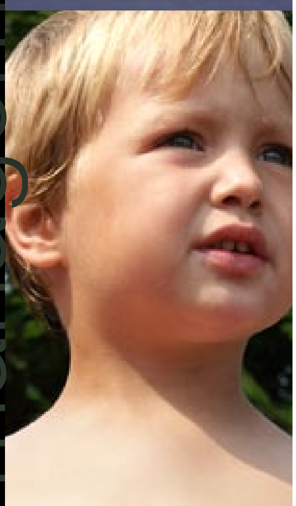
Introduction of Predatory Fish

Control of mosquitoes often includes introduction of minnows, such as Gambusia affinis (mosquito fish), or a Colorado native, the plains killifish (Fundulus zebrinus). The top-water feeding minnows can consume approximately 100 mosquito larvae per day when they become adults. Their offspring begin feeding immediately after birth and they are able to have several gestations per season. Similar to Bti and Bs, stocking of predatory fish is considered a biological control. However, fish are less species-selective than are the two species of bacteria. Fish introduced to a wetland will eat mosquito larvae as well as all other palatable aquatic life in the wetland potentially having detrimental effects to nontarget species.

Application of Oils or Monomolecular Surface Films

The application of oils to water is not species specific (EPA 2002). Products containing mineral oil such as Bonide Oil or Golden Bear Oil, or a monomolecular surface film such as Agnique are allowed for limited use only approved on a case-by-case basis by the Colorado Division of Wildlife and the Colorado Department of Public Health and Environment (CDPHE). Oils or surface films are used to mainly treat mosquitoes in the pupal stage. Gilled aquatic insects are apparently not affected by oil treatments, but they are lethal to most surface-breathing aquatic insects or those that depend on a breathing tube. The oil causes them to suffocate. The monomolecular surface films are effective by reducing surface tension on the water, which prevents larvae or pupae from hanging from the surface. This action causes them to drown. There is also the potential for flying insects that land on the water to be impacted, but this has not been study comprehensively.

These alternatives are not recommended for use by the city of Boulder, as they could severely affect the biodiversity and abundance of aquatic life within a wetland as well as alter the aesthetic appearance. These types of treatments must be closely monitored for effectiveness of eliminating the pupae and for affects to nontarget species.



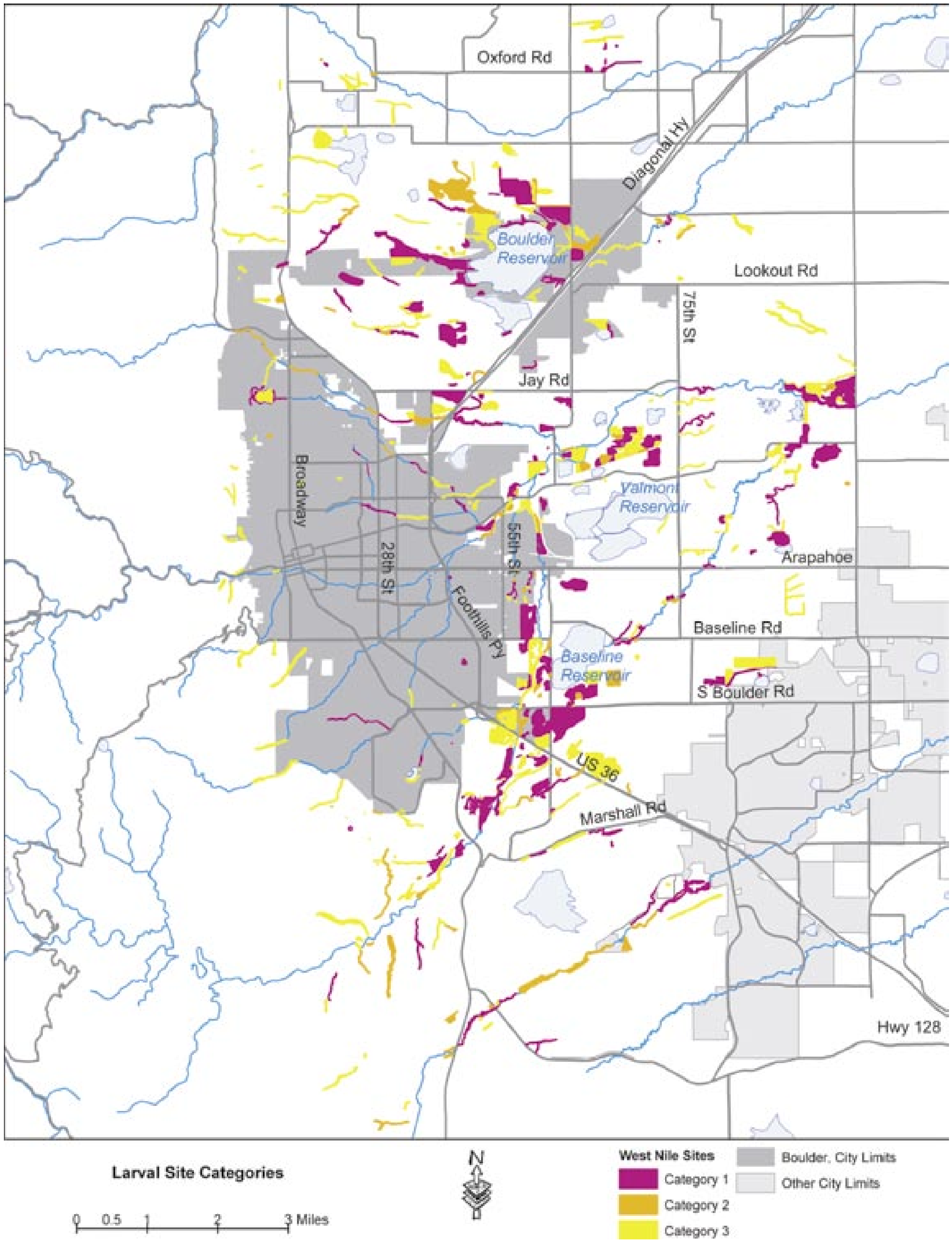


Figure 2-1 Larval Breeding Site Locations and Categorization

Larval Mosquito Monitoring Plan

As of 2005, a majority of potential larval breeding sites have been identified, surveyed, and mapped. Not all potential habitats necessarily breed mosquito larvae and further, not all habitats that breed mosquito larvae produce Culex. However, certain sites have bred Culex larvae consistently when surveyed or received one or more Bti treatments. Therefore, it makes sense to differentiate between habitats and focus initial surveillance efforts only at the sites where Culex mosquitoes have historically occurred and/or where Culex mosquitoes are more likely to occur at some point during the season. Based on data collected from 2003-2005, 488 sites have been categorized as follows:

Category I - Vector larval breeding sites: All sites where Culex larvae have been found breeding

Category II - All mosquito larval breeding sites: All sites where only nuisance mosquito larvae have been found breeding (to date)

Category III - All potential larval breeding sites: All potential mosquito breeding sites that have not been found breeding any type of mosquitoes (to date)

In general, Boulder's mosquito season occurs May through September, while the peak season for Culex breeding is June through August. Given this ecology, the following is a table of when and where mosquito management will occur, assuming a 'normal' mosquito year:

Management Actions	May	June	July	August	September
Category I Vector larval breeding sites	X	X	X	X	X
Category II All mosquito larval breeding sites		X	X	X	
Category III All potential larval breeding sites			X		

Table 2-2 Annual Baseline Larval Mosquito Monitoring and Control Plan

Larval monitoring and needed control will occur at all sites where Culex larvae have been found, during any of the three previous years of monitoring, once per week during the entire mosquito season. Monitoring and any necessary control will also occur, once per week, at all mosquito larval sites that have ever been found to be breeding non-Culex mosquitoes during the height of the Culex breeding period (June-August). Monitoring and any necessary control at all remaining mapped potential larval breeding sites within or near city limits will occur once during the peak season (beginning in the first week of July) as a measure of quality control. Weather will also determine the frequency at which sites will be monitored. If a site is found to be dry, that particular site will not be checked again until it rains or is irrigated.

As the season progresses sites may be moved to other categories. For instance, if Culex is found in a Category II site, that site will be treated and redesignated as a Category I site for the remainder of that season and for following seasons. Similarly, if non-Culex mosquito larvae are found in a Category III site during the check in early July, it will be redesignated as a Category II site and checked more frequently for the remainder of the season and will remain a Category II site for the following seasons.

The above plan is designed for maximized vector control, as pre-peak and post-peak season Culex larval control will likely reduce Culex adults from emerging later in the season or even the following year (Culex can overwinter). Monitoring and control in Category II sites during the heightened Culex breeding period and in Category III sites during peak Culex activity (as particular precipitation and temperature conditions could produce sites hospitable to Culex in places where they have not yet been observed) will further enhance WNV prevention and early detection and provide quality control as a measure of success in detecting fluctuating sources for Culex.

Thresholds for Increased Larval Vigilance and Management

The plan also allows for maximum flexibility in monitoring and control, should a combination of thresholds be met at any point in the season that may warrant increased vigilance. Thresholds for increased monitoring between site categories include:

- A significant percentage (~50%) of vector sites that are breeding Culex larvae in May,
- Early detections of WNV in area adult sentinel traps,
- High or rapidly increasing adult mosquito populations in general, and
- Early or rapidly increasing cases of WNV in humans and/or birds.

Below is the same table that demonstrates what increased management actions may be taken in a high mosquito and/or WNV year, where _ represents an above average to high mosquito year and _ indicates an WNV epidemic year (such as in 2003).

Management Actions	May	June	July	August	September
Vector larval sites	X	X	X	X	X
All mosquito larval sites	_	X	X	X	_
All potential larval breeding sites	_	_	X	_	_

Table 2-3 Increased Monitoring and/or Management Plan for Larval Sites

Larval Monitoring Protocol

To inspect a project site a plastic dipper cup with a 3-foot wooden handle is used to collect water from the site. Each sample (dip) is closely examined for mosquito larvae presence. If mosquito larvae are present, an eyedropper is used to collect a representative sample for future species identification. A representative sample consists of mosquito larvae with all the various instars (life stages) that are present. The majority of the sites have poor open water habitat in the center and good habitat around the perimeter of the site. At these sites, the dipping effort is completed using a linear approach (walking around the perimeter and sampling the margins). Some sites are small (1 acre or less) and have good habitat throughout the site. At these sites the dipping effort is completed using surface area guidelines where the entire site is methodically sampled.

Using the linear approach, sites 1 acre in size and less are dipped approximately every 10-20 feet; sites 1 to 10 acres are dipped approximately every 50 to 100 feet, and sites greater than 10 acres are dipped approximately every 200 to 500 feet. Using the surface area approach, sites 1 acre in size and less are dipped approximately every 10 to 20 square feet. Since each project site varies in size, physical characteristics, and changes as the season progresses (e.g., becomes drier, wetter, increased vegetation), there are field adjustments made during the season concerning appropriate number of dips. For example, at a very small site (less than 1/10 acre), the site may be dipped every 1 square foot to get good coverage. At the beginning of the season, all sites are monitored consistently. During the peak season (July through August), the sites are categorized into high and low priorities due to heightened risk of WNV and time effectiveness.

Larval Control Protocol

Larval mosquito control methods employed by the city of Boulder are designed to reduce the potential of WNV. The program's focus for larval control is to identify if Culex species (primarily C. tarsalis and C. pipiens) were present before initiating control efforts. The threshold for larval control is presence of the vector species. The method allows for pest mosquitoes and non-biting mosquitoes to persist in the environment if vector species are not present. The approach requires more frequent monitoring but results in substantially less treatment of (and therefore less potential adverse impact to) wetlands and other mosquito breeding habitats. Unlike a large-scale broad-spectrum mosquito control program, where presence of any type of mosquito larvae triggers treatment, the presence or absence of vector species has to be determined before site-specific larval treatment occurs. Finding and documenting consistent vector breeding sites is an important component for the program because it creates a pattern that can be monitored and systematically controlled. The objective of larval mosquito control is to prevent the need for adult mosquito control spraying, which is much less effective than larval control.

To accomplish this more effective protocol with as little impacts as possible, it is necessary to have a team more skilled than is typically necessary for mosquito control efforts. It is likely that the contracted service that performs the monitoring and control of sites will need to maintain a strong biological staff during the season including a core team of professional aquatic biologists, wetland scientists, and mosquito control specialists, all with aquatic invertebrate identification skills. This core team should likely be supported with

LARVAL SURVEILLANCE FLOWCHART

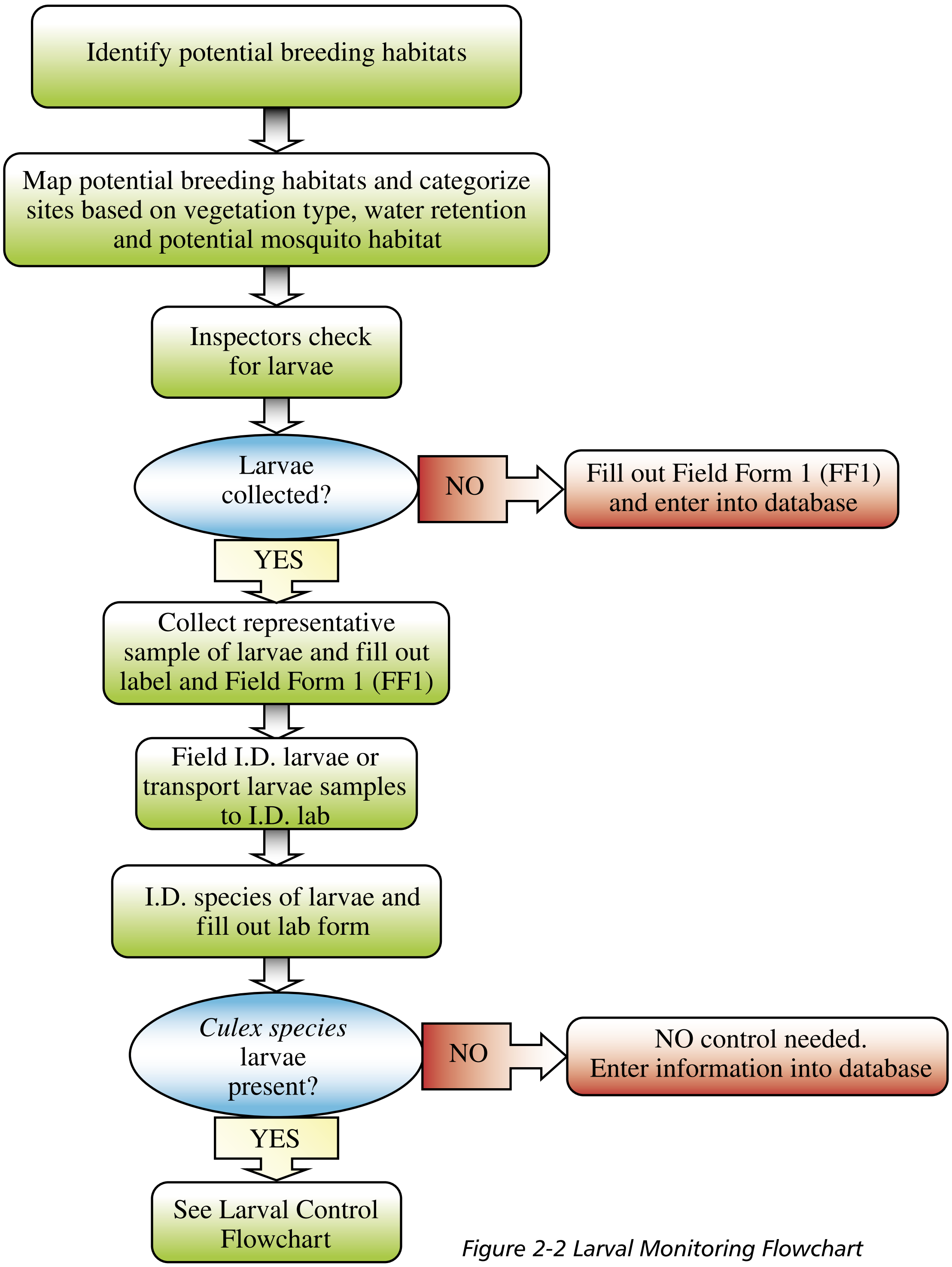
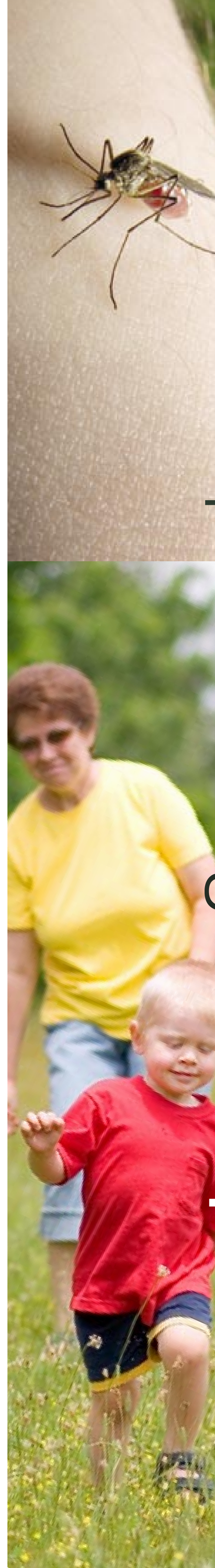
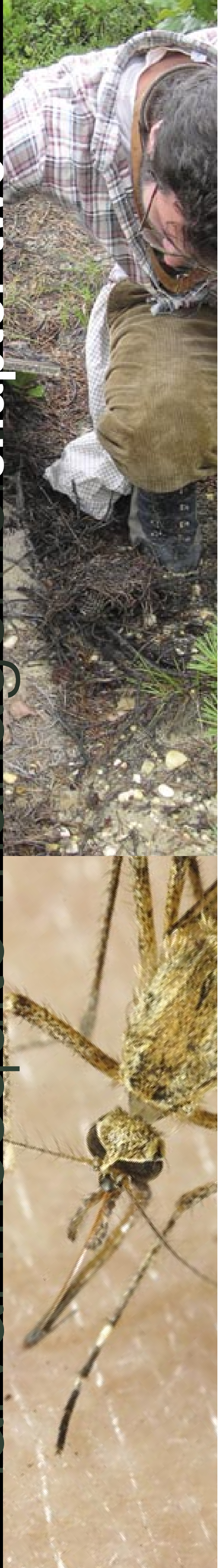


Figure 2-2 Larval Monitoring Flowchart

a seasonal crew with natural resource and biology backgrounds and experience in mosquito larvae and adult monitoring/control. The contractor and/or city of Boulder should use geographic information systems (GIS) ArcView software to compile wetland areas, mosquito habitat, adult trapping locations, etc. to aid the larval mosquito monitoring and control creating a database for quick access and queries.

A city wetland permit is needed for larval control when using a pesticide. See Appendix C for a copy of the current wetland permit and procedures for renewal.





Larvicide Application Methods

The application of Bti is the approved method for larval mosquito control because it serves the greatest public need. Adverse impacts to areas being treated are minimized by applying Bti at the recommended concentrations, and all treatment areas are posted with bright yellow city pesticide notification signs before Bti is applied.

The usual application rate used for Bti is 5 pounds/acre or 0.2 acres treated per 1.0 pound of Bti. The application rate seems to provide 100 percent control in the majority of treatments. Applicators use appropriate personal protection equipment (PPE) when applying the Bti including gloves and filter masks.

Larvicides are applied in accordance with regulations set forth by the city of Boulder and other governing bodies. Recommendations for applying larvicide are provided to city officials and include the type and amount of material to be used on a case-by-case basis. Larvicides are applied by ground crews where necessary. Currently, there is no plan to apply larvicide with aerial equipment. Larvae are collected after treatment to evaluate the efficacy of control operations.

LARVAL MOSQUITO CONTROL FLOWCHART

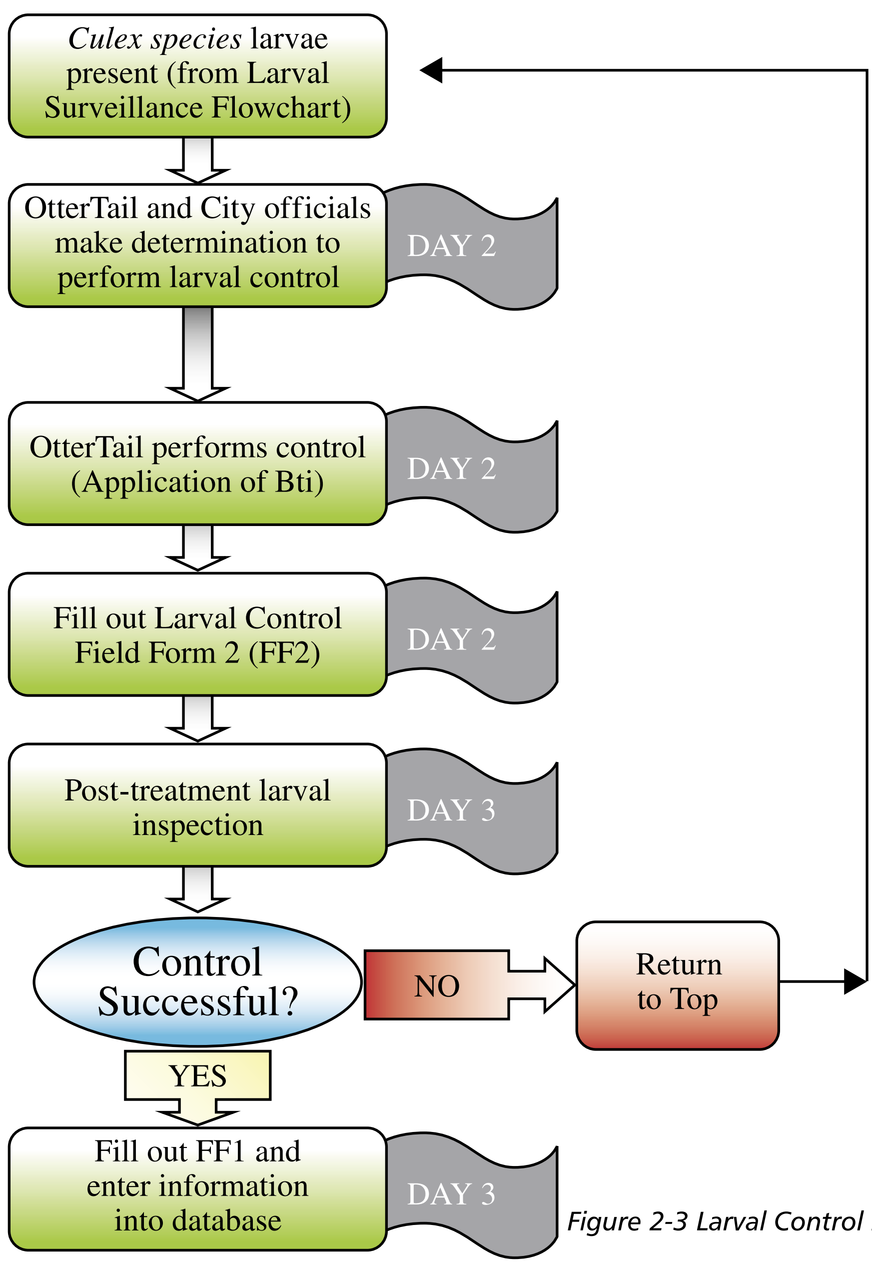


Figure 2-3 Larval Control Flowchart

Bti Handling and Spill Control

Bti is spread on treatment areas by hand or by using hand seeders and power spreaders. When a site is identified as requiring treatment, the seeders are filled with the appropriate amount of control material, as indicated on the label, is measured out for the site and then applied. Field personnel wear appropriate protective gear, including goggles, gloves, dust masks, and long sleeved clothes. Seeders and spreaders are filled in the field vehicles to minimize the chance for spills.

In the event of a spill of controlled material (Bti) that could result in significant injuries, significant property damage, or environmental threat, the following guidelines are followed.

- Report any spill immediately to the field crew leader
- Be familiar with the spill response kit
- Confinement and cleanup of material

1. Avoid direct contact with skin and avoid dust inhalation
2. Recover the product, place into appropriate container for disposal
3. Ventilate and wash the spill area, if necessary
4. Dispose of product in accordance with federal, state, and local regulations

- Notify city staff and other agencies as appropriate

CHAPTER 3: ADULT MOSQUITO MANAGEMENT

Adult Mosquito Monitoring Plan

Proper monitoring of adult mosquitoes, which includes testing for the presence of WNV, is important in guiding prevention and control because it can provide information on the potential threat to residents and can indicate areas where efforts to eliminate mosquitoes should be targeted. At present there are 23 trap locations set up within and near city limits; 16 are checked and maintained by city of Boulder contractors, 4 by Boulder County (or its contractor), and 3 by University of Colorado. Figure 3-1 shows approximate adult trap placement around the city. The following table shows the city's plan for checking its traps during mosquito season:

Management Actions	May	June	July	August	September
Adult trap monitoring (16 traps)	*	X	X	X	*

Table 3-1 Adult Mosquito Monitoring Plan * indicates other agency monitoring

All city traps are monitored weekly for WNV in adult mosquito populations during the peak of Culex activity. The adult monitoring plan does not cover the entire length of mosquito season (or potential Culex breeding period), though forewarning of especially early or late season Culex activity in adult traps can be gathered from contacting the CDPHE and BCPH on a regular basis (BCPH usually maintains a total of 41 traps within its mosquito control district). City of Boulder staff or its contractor may begin monitoring its adult traps for WNV presence earlier (May) or later (September) than planned as shown above if data from partner agencies indicate that there are early, rapidly increasing, or high sustained levels of vector mosquito populations and/or early, elevated, or sustained cases of WNV present in humans and/or bird and mosquito populations. A continued and extensive communication network will serve as the best resource to make the most informed decisions on monitoring and control of WNV. This network involves city agencies, CDPHE, BCPH, Colorado University, regulatory agencies, and other bodies associated with monitoring of WNV.

If areas are found to support adult Culex carrying WNV, additional traps may be set in those areas. Sweep net samples may also be taken in areas where large numbers of adult mosquitoes are observed to supplement the trap data.

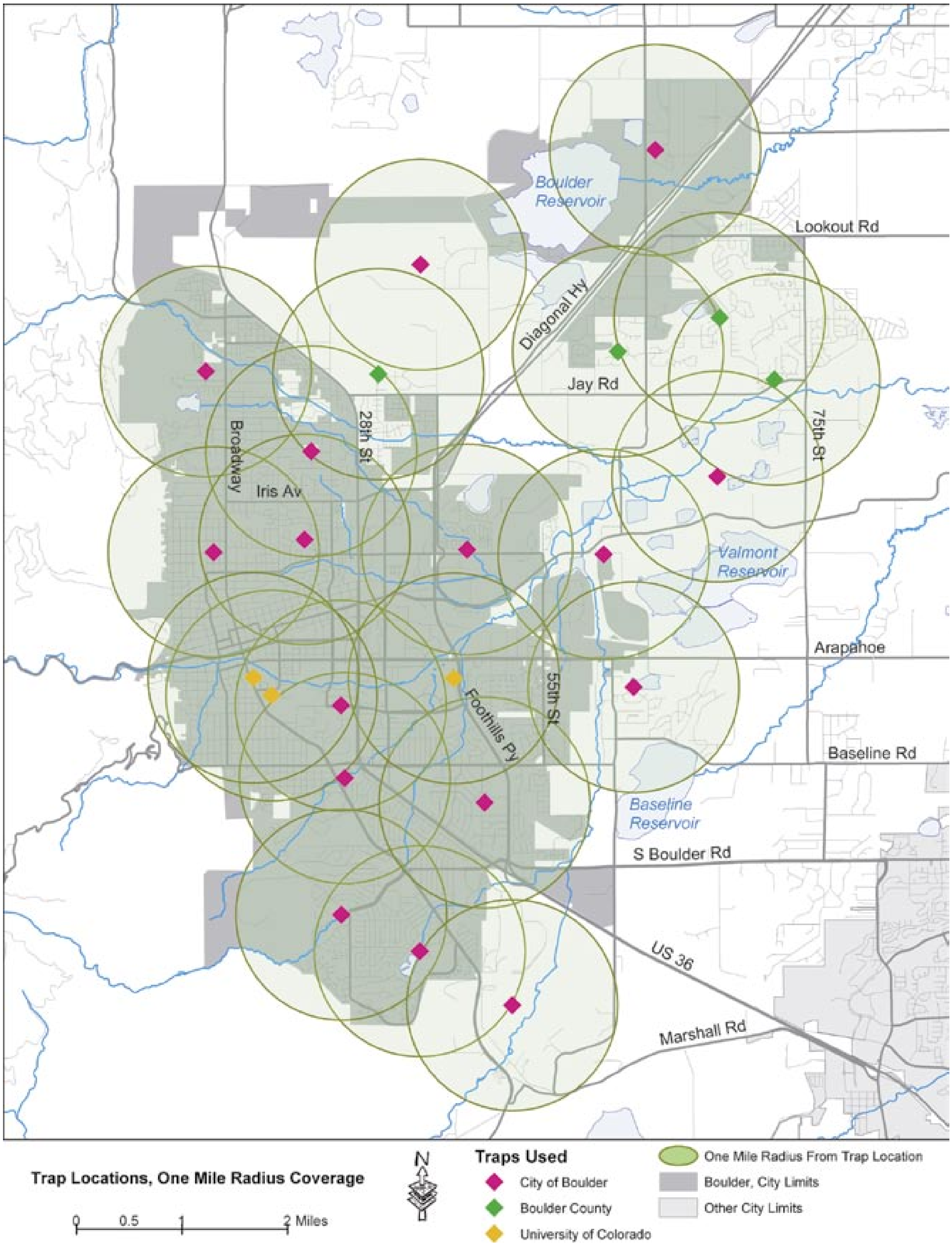


Figure 3-1 Adult Mosquito Trap Locations

Adult Mosquito Monitoring Protocol

Trap Site Selection

Adult mosquitoes can dehydrate quickly during the daylight hours if they do not have a shady area to rest and escape the heat. Most mosquito species prefer to rest during the heat of the day in areas known as harborage areas. A mosquito harborage area is usually a shaded, wind-protected, moist area that is often near a source of water. Harborage examples include groves of tall trees with a layer of shrubby undergrowth, dense bushes, tall live grasses, or under roof eaves and inside tires in residential areas. Adult mosquitoes will even collect in shorter grasses that are lush from frequent watering.

The harborage sites for the project are important for long-term monitoring; therefore, the trap sites were chosen carefully. Staff worked with the BCPH to identify 16 adult trap locations and the radius around each trap that could potentially be sprayed. The city cooperates with the University of Colorado and Boulder County in order to provide complete coverage for the city. The locations of the adult mosquito traps were chosen based on providing thorough coverage of the entire city as well as cover areas where human cases occurred in 2003. A recommendation from the CDC is that adult traps not be placed close to larval breeding sites because newly hatched mosquitoes have not had a chance to become infected with WNV; therefore a lower infection rate may be obtained from the data than is actually present in the mosquito population.

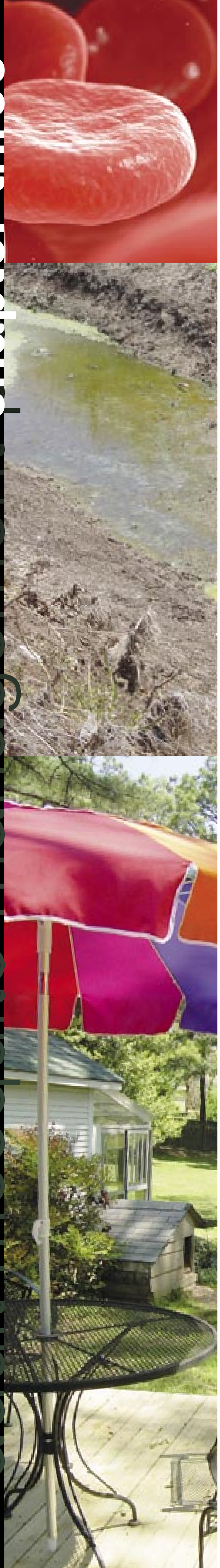
Trap Methods

There are two different types of adult mosquito traps typically used to monitor adult populations, the gravid trap and the carbon dioxide (CO2) light trap. The CO2 light trap is used to capture a representative sample of the types and numbers of an area's adult mosquito population. The gravid trap attracts adult female mosquitoes that have already collected a blood meal and are ready to lay their eggs. Both types of traps have been incorporated into the city of Boulder's WNV monitoring program.

To capture the most representative sample of adult mosquitoes in an area, adult CO2 light traps are set overnight to collect live adult mosquitoes throughout the season. The traps are based on the principle that most adult mosquitoes are attracted to light, CO2, and heat. The CO2 light trap collects adult female mosquitoes that are attracted to mammals. Therefore, the CO2 trap indicates when the vector mosquito species are no longer feeding solely on birds and have turned to mammals as their alternate blood meal. This is one of the first indicators that WNV is likely to be transmitted to people through the vector mosquito (assuming the trapped vector mosquitoes are found to be carrying WNV).

The trap consists of a plastic insulated bucket with a battery casing hanging directly below the bucket and a finely meshed net hanging below the battery at the very bottom. The entire device is placed on a tree branch about 5 to 7 feet off the ground by a small chain or rope letting the bucket and net hang free. The bucket is filled with 2 to 3 pounds of commercial dry ice (CO2) and holes at the base of the bucket allow a slight amount of CO2 to leak out as an attractant. The battery runs a small fan and light positioned above the net. The light provides further attraction and once the mosquitoes are near the light, they are sucked down into the net and trapped by the downward force of the fan. In the morning, the mosquitoes are removed and then frozen to aid in identification. Once identified, the mosquitoes are then sorted by species and vector mosquitoes are submitted to CDPHE for WNV testing.

The gravid trap mimics sources of mosquito breeding habitat and collects gravid female mosquitoes. The trap consists of a plastic tub with a fan unit and net above it. The device is placed on level ground in a mosquito harborage area. The plastic tub is filled approximately halfway with an odorous attractant that is obtained from a hay infusion (a fermented mix of water, manure, and hay that mimics stagnant, organic water that is highly



attractive to gravid female Culex mosquitoes, particularly C. pipiens). Above the tub is a fan unit with a finely meshed net attached to it. As the mosquito flies along the surface of the attractant to lay its eggs, it is sucked up by the fan and into the net. The net is collected the next day and the mosquitoes are frozen to aid in identification. Once identified, the mosquitoes are then sorted by species and vector mosquitoes are submitted to CDPHE for WNV testing.

Pooled Traps

Because of limited resources of all area agencies involved in WNV management, vector mosquitoes from the CU and the city of Boulder adult traps are pooled before they are submitted to the CDPHE for testing. It is expected that this method will be used throughout the season until a pooled sample proves positive for WNV. In this case, traps will then be submitted and tested individually during the next trapping cycle in order to try to determine a general area where WNV may occur.

WNV Detection

Initial or a single detection of mosquito-borne pathogens in mosquito or avian populations within Boulder will be communicated to the City Manager. When approved, a press release will be drafted to notify the public of the recent findings. Monitoring will continue with the following added activities:

1. Adult mosquito trapping will begin, or will be increased, in the area of concern if additional monitoring data are required.
2. Larval monitoring will be enhanced in affected areas if needed.
3. Laboratory testing of adult mosquitoes will be a priority in affected areas.

Data from these additional collections will aid in evaluating the extent of pathogen transmission and mosquito populations and be used to guide control measures, if applicable.

In addition to the above actions, persistent detection of WNV in vector mosquitoes or in non-avian vertebrate populations, such as horses, within Boulder (County) will prompt the following control measures to be considered:

1. Ground application using backpack applicators of adulticides to immediate areas of concern in harborage areas where large numbers of adult mosquitoes are concentrated would be recommended. Truck-fogging would not be recommended in these areas.
2. Application by truck-mounted fogger of adulticides to broader areas, based on monitoring data.

Adult Mosquito Control - Contingency Management

Adult mosquito control agents (adulticides) are materials applied for control of adult mosquitoes. Control of adult mosquitoes is not an assumed part of this plan, but is included as a contingency measure if monitoring suggests that the level of WNV activity poses a significant threat to human health. The products that would be used in this event are discussed below.

The data on spraying for adult mosquitoes shows a range of efficacy, from 10% to 80%, depending on the amount of vegetation, environmental factors, and the mosquito species. Entomologists at the Center for Disease Control (CDC) report that the percent reduction in Culex populations in recent studies is 30-35%. Other mosquito species are killed at higher rates. Most communities and mosquito control districts that conduct spray programs use the number of mosquitoes as a guide in determining spraying needs. City of Boulder staff coordinated BCPH and the CDC to refine a spray protocol for the city of Boulder for an emergency adult vector mosquito spraying program.

ADULT SURVEILLANCE FLOWCHART

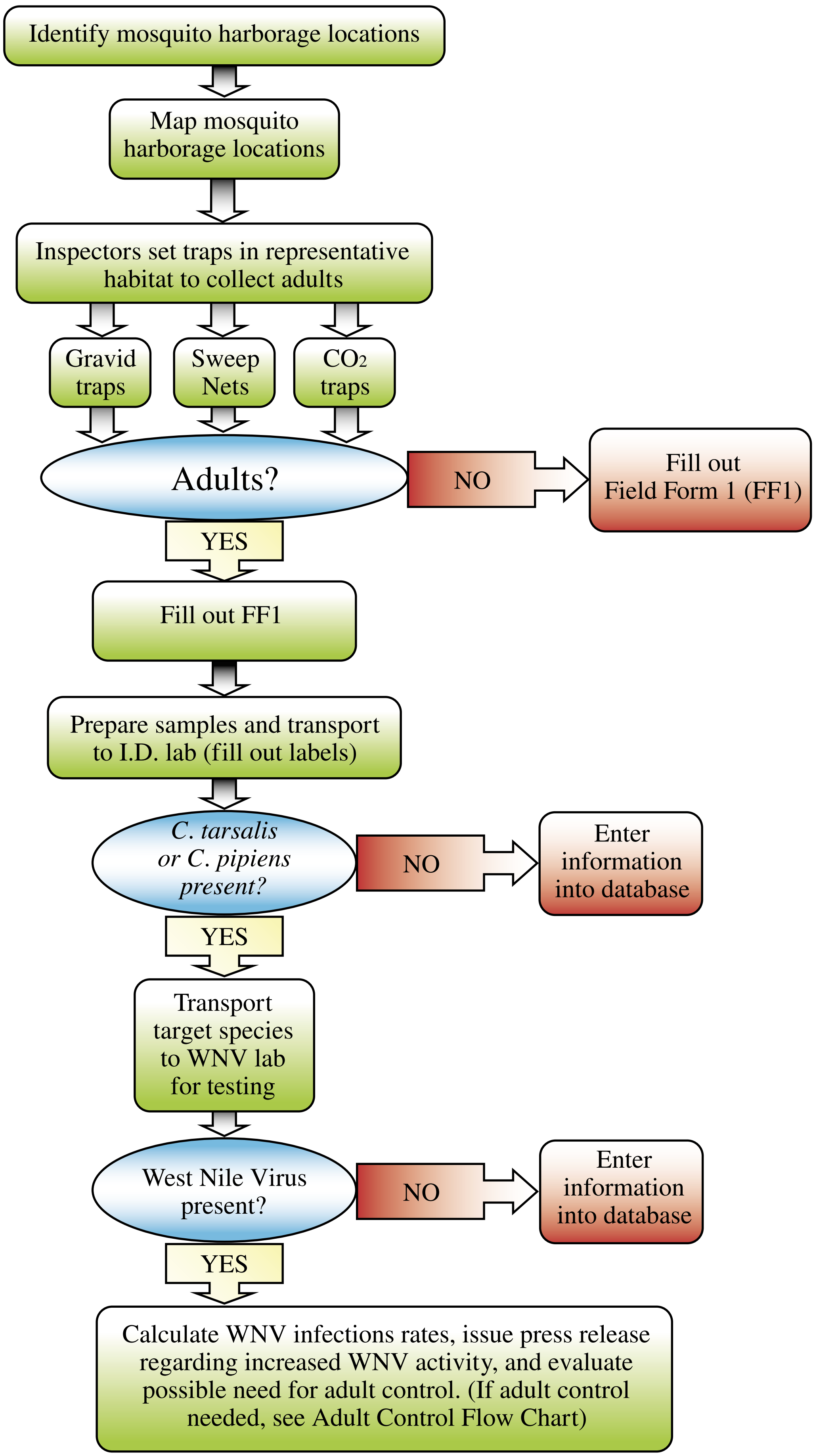


Figure 3-2 Adult Mosquito Monitoring Flowchart

Preferred Adult Control Agents

The adulticides recommended for application under the adult mosquito control contingency plan are known as synthetic pyrethroids. Synthetic pyrethroids are synthesized derivatives of naturally occurring pyrethrins, which are taken from pyrethrum, the oleoresin extract of dried chrysanthemum flowers. The three specific pyrethroids that could be implemented as a contingency measure for adult mosquito control are permethrin, resmethrin, and sumithrin. A label and MSDS sheet adult control agents can be found in Appendix B.

Potential Impacts of Synthetic Pyrethroids

These products cause rapid knockdown of adult mosquitoes and are typically mixed with a synergist compound, such as piperonyl butoxide, which enhances the effectiveness of the active ingredient. They exhibit low mammalian toxicity, degrade rapidly in sunlight, leave little or no residue, and do not bioaccumulate in the environment. Dosage rates can be low to control mosquitoes. These products are applied in small quantities per acre, referred to as ultra-low volume (ULV) application. ULV delivery techniques minimize environmental impacts at the same time they effectively manage populations of adult mosquitoes. The city or its contractor will monitor the application of the adulticide to ensure compliance with all regulations and requirements stated by the EPA, State of Colorado, and city of Boulder.

Synthetic pyrethroids are effective in killing mosquitoes, gnats, biting and non-biting midges, black flies, and other biting flies. These insecticides readily bind to soil and other organic particles; however, they are degraded by sunlight in water and on soil surfaces. Pyrethroids are extremely toxic to honeybees. Product labels specify requirements for minimizing effects to bees (Bayer Environmental Sciences [BES] 2002), though care should be taken to not apply these products in crops or weeds where foraging by honeybees may occur (BES 2002b).

According to the EPA, Pyrethroids can be used for public health mosquito control programs without posing unreasonable risks to human health when applied according to the label. However, they are considered to pose slight risks of acute toxicity to humans, and at high doses, pyrethroids can affect the nervous system. According to the Center for Disease Control, People who are concerned about exposure to a pesticide, such as those with chemical sensitivity or breathing conditions such as asthma can reduce their potential for exposure by staying indoors during the application period (typically nighttime). Pyrethroids are extremely toxic to aquatic organisms; however, recommended manufacturer dosage rates control the toxicity of these products to non-target species. Lobster, shrimp, mayfly nymphs, and zooplankton are the most susceptible non-target aquatic organisms (Mueller-Beilschmidt 1990). Some permethrin based mosquito control products direct the user not to apply the product within 100 feet of lakes or streams. This restriction or "buffer zone" was put on many permethrin labels out of concern for aquatic toxicity that might result due to runoff from agricultural sites, not as a result of an assessment of risks associated with the significantly lower concentrations of the active ingredient involved in ULV mosquito control applications. Resmethrin product labels state "Avoid direct application over lakes, ponds and streams" (emphasis added), but the same labels state that vegetation "around stagnant pools, marshy areas, ponds and shorelines may be treated" and there is no buffer zone requirement (EPA 2005).

Thresholds for Initiating Adult Mosquito Control

Adult control activities that include spraying of pesticides will be the final option to pursue in an attempt to reduce the threat of WNV. Spraying of pesticides in an urban environment is the most costly, inefficient, and environmentally unfriendly procedure in an IMM (Integrated Mosquito Management) program. Responsible IMM practices include extensive larval control with a contingency plan for adults. Theoretically, the ideal larval control plan would eliminate the need for any control of adults. However, the rapid development of mosquitoes from egg to adult and the persistent nature of breeding in an extensive variety of stagnant water bodies make complete elimination impossible. The flight of C. tarsalis can range as far as 20 miles. Although it is rare for C. tarsalis to fly 20 miles, it is possible that mosquitoes outside the city limits would migrate into Boulder. Therefore, a plan for control of adults is necessary as a contingency.

Adult control thresholds for disease vectors are much harder to establish and justify compared to larval thresholds. Because the adult mosquito is no longer in a contained aquatic environment, adult control is not nearly as effective. Adult mosquitoes will often vacate an area until the adulticide evaporates or settles to the ground. Adulticides also have more impact on non-target organisms compared to larvicides.

Final recommendations on adult control activities will weigh heavily on recommendations from the city of Boulder management staff, CDPHE, BCPH, CDC, and EPA. Among the thresholds established for implementation of adult mosquito control activities is finding WNV in C. tarsalis or C. pipiens adult females. Meeting or exceeding these thresholds does not directly correlate to spraying pesticides every time WNV is discovered in adult mosquitoes. Control for adult mosquitoes will be determined on a site-specific basis.

The presence of mosquito-borne pathogens in Boulder, if detected, will prompt one or more responses or interventions. These interventions can range from continuing existing routine monitoring to, in worst-case scenarios, large-scale application of adulticides. Monitoring data will be used to assess the risk of an outbreak of human disease and the need to apply pesticides in a limited and targeted area to control adult mosquitoes. The control response will depend on a combination of thresholds being met that include, but are not limited to:

- the overall intensity and persistence of the WNV activity in adult Culex mosquitoes, humans, birds, and non-avian vertebrates
- the proximity of WNV activity to human populations within the city
- the time of year
- vector index level
- seasonal climate

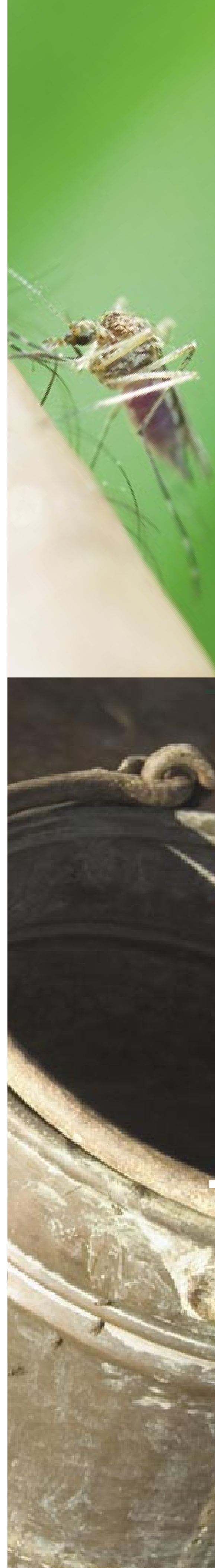
Explanation of Mosquito Infection Rates and the Vector Index

The CDC encourages surveillance programs to routinely incorporate a more informative index of relative virus activity, the virus infection rate (IR), into their mosquito-based evaluation of local virus activity patterns. At the county level or below, weekly tracking of mosquito IR can provide important predictive indicators of transmission activity levels associated with elevated human risk.

Estimates of the IR are usually presented as the number of infected mosquitoes per 1,000 tested. The model used by the city of Boulder to calculate the virus infection rate, is called the Maximum Likelihood Estimation (MLE) and is the model approved and being used by public health officials and epidemiologists across the country, including the Boulder County Public Health Department.

An index, based on the vector MLE and mosquito populations, was applied to the 2003 human WNV case number data by OtterTail and the City of Boulder to determine a single number that would indicate the potential need for adult mosquito control measures. During this process, they determined that 0.75 would have provided an early warning of the pending human health epidemic (See Figure 3-3). To calculate the index, the number of Culex species mosquitoes is multiplied by the calculated WNV infection rate of the vector mosquitoes. OtterTail and the City's proposed index rate of 0.75 was discussed with CDC and BCPH staff and approved by the Boulder City Council as the critical level that would trigger adult mosquito control.

The index was developed by combining the data for all the traps in 2003. Although it would have been preferable to use this on an individual trap basis, this was not possible in 2004 due to the mathematical inability to calculate infection rates on one sample. Therefore, the index was used simply as a city-wide early warning system based on the average of all the city traps. The index is now being used, in combination with other thresholds, as an additional tool to aid in the decision making process for the possible need of any adult control activities.



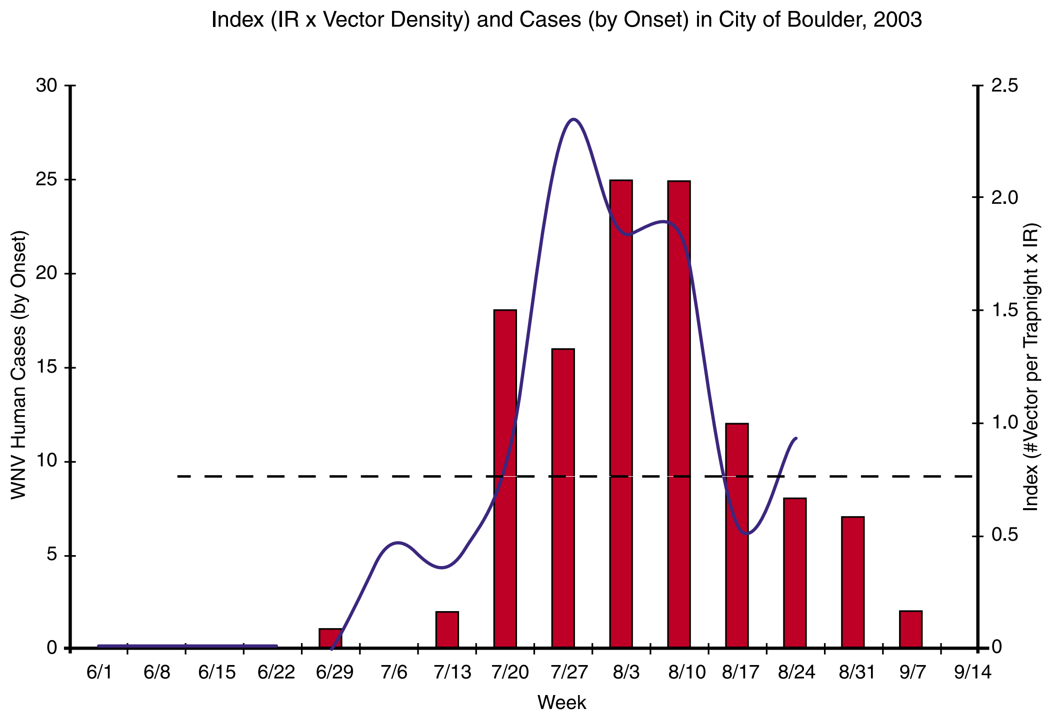


Figure 3-3 Vector Index

Adult Mosquito Control Protocol

There are two general strategies for mosquito adulticide applications, large-scale fogging and spot spray treatment. The first and most expensive option is to fog large areas to attempt to significantly reduce the number of adult mosquitoes in a targeted area. The effort could include many types of application methods: ground individual backpack units, foggers on ATV's, foggers on trucks, and aerial applications. To use only one method, such as fogging with a truck-mounted unit, would be far less effective than using multiple methods. However, a full-scale assault could undoubtedly reduce the adult mosquito populations but the process could use an entire season's budget in less than a month. Also, the main purpose of a large scale fogging approach is to keep the adulticide material airborne as long as possible without it evaporating. To keep the adulticide airborne a certain droplet size is needed and only expensive adulticiding equipment can do this accurately. The effectiveness of the large scale fogging effort will be compromised if the control company does not have somewhat of free range of the city area to perform control. Public concerns or the majority of residential areas on a no-spray list will make effective, widespread control almost impossible.

The second option is to spray individual harborage areas, which is called spot treatment. This was the method used at the Boulder Reservoir in 2003 (the only adult spraying event that has occurred for WNV to date in the city of Boulder). Treating the harborage areas where adult mosquitoes are resting in high concentrations allows more mosquitoes to come in contact with the adulticide and uses a lower, focused amount of adulticide material. Each harborage area has unique characteristics and circumstances. Some areas can be controlled effectively with manual removal of the mosquito harborage habitat such as mowing long grass and removing vegetative understory; other areas can only be controlled effectively by spot spraying adulticides. After treatment a post check adult collection is performed to determine effectiveness.

Spot treatment has many positive aspects. First, it helps to identify possible overlooked mosquito breeding sites and areas where larval control should be focused. Secondly, spot treatments are (publicly) lower profile than large-scale operations with much less adulticide material being used in the environment. Also, spot treatment potentially kills the same or more mosquitoes than fogging because the mosquitoes are concentrated in smaller areas.

Adult mosquito control would occur in the evening when mosquitoes are most active with hand-held spray units and truck-mounted fogger units.

WNV No-spray List

City residents may opt-out of the adult mosquito spraying program. The city of Boulder 's Office of Environmental Affairs maintains a database of city residents who have called, emailed, or written letters requesting that their property be skipped if adult control is necessary in their immediate area. A list of registered addresses will be provided to the contracted service, who will in turn avoid spraying within 50 feet of the parcel boundaries. In many instances, this no-spray buffer affects neighboring properties, regardless of their interest in participating in the adult control program.

As of 2005, 586 private parcels are registered on the no-spray list. In some areas, particularly more densely occupied residential areas within the city, the no-spray zone covers a majority of a block or neighborhood. This presents real challenges for control, should a WNV event occur nearby that necessitates adult spraying.

Each year, notification cards are sent to registered addresses notifying them that their address is included on the no-spray list. No action is necessary for them to remain on the list from year to year. However to remove an address from the no-spray list (and become part of the spray program), residents must call the city's WNV hotline (303-441-4004) or email the city's WNV program by visiting www.environmentalaffairs.com.

Precautions and Notification Prior to Control

There may be restrictions on or cancellation of planned outdoor evening activities or recommendations to close recreational areas. The public will be notified 48 hours in advance of the schedule for application of adulticides, potentially through reverse 911 calls in the affected area, which will allow time to take any necessary precautions to reduce exposure to the pesticide. An informational brochure will be distributed to the public who reside within a 1/2 to 1 mile radius of the epicenter where the WNV pathogen is detected. The brochure will contain recent findings and protective measures to be taken.

Before control of adult mosquitoes begins, information on pesticides and their possible adverse health effects will be provided to physicians and other health care providers, and hospital emergency departments. This information will include product information on pesticides, Material Safety Data Sheets, and other information relevant to identifying possible exposure to pesticides. Calls received by the Colorado Poison Control Center will continue to be monitored during pesticide spraying and will be forwarded to the Colorado Registry for Pesticide Sensitive People for possible follow-up and inclusion in the registry.

Information will be distributed at least 24 hours in advance through the media, the city website, IPM hotline, and pertinent city and community organizations.

Control measures will be monitored and assessed for potential environmental and health effects through several means, including pre-application and post-application environmental sampling and complaints of exposure to pesticides received. Sampling will include pre-treatment and post-treatment checks using adult mosquito traps. Specimens will be collected more frequently to evaluate the efficacy of the control measures.

Example of Adult Mosquito Spraying Schedule

All traps will be set on Tuesday night and checked Wednesday morning. Based on information from multiple thresholds, city officials would make the decision that emergency adult control is needed. Among the thresholds observed, in this example, are unusually high trap populations and infection rates from one of the traps. Therefore, it is determined that a one-mile radius would be sprayed around this trap. Two cycles of spraying would automatically occur, one on Monday and one on Thursday (the night of the third day) as recommended by the CDC. If the thresholds are still being met, including the information in the data from the post-spraying mosquito trapping, another spray event (two cycles) would occur in the next week. If the combined thresholds suggested that the threat of WNV was still high, spray events would continue until the threat levels fell.

Timeline Example:

Wed: Mosquito trap collected and mosquitoes sorted by species and sent to State of Colorado Laboratory for WNV testing.

Fri: Data received from lab and infection rate and index calculated. Data from multiple thresholds lead the city to decide that an emergency adulticide application will take place; the following actions would be initiated:

Sat: Staff contacts spray contractor and confirms date for spraying (in the evening two days away). Residents in trap area notified, potentially by 911 callback, press release is sent out and information posted on city's website.

Sun: Notification continues.

Mon: The spraying would occur in the evening, assuming that environmental conditions are favorable for spraying (i.e. low wind speed, no precipitation). Pick-up trucks with ultra-low volume (ULV) sprayers would drive the streets in the designated area. They would have flashing lights and would be identified as mosquito control equipment. The contractor will have the addresses from the city's "no spray list" and will shut off the sprayer upwind of these addresses.

Tue: Post-spray adult mosquito trapping. Notification repeated for spraying on Day 9 (Thursday).

Wed: Continue notification.

Thu: The spraying operation described for Day 6 would be repeated.

Fri: Post-spray adult mosquito trapping.

Wed: Regularly scheduled adult mosquito trapping.

ADULT MOSQUITO CONTROL FLOWCHART

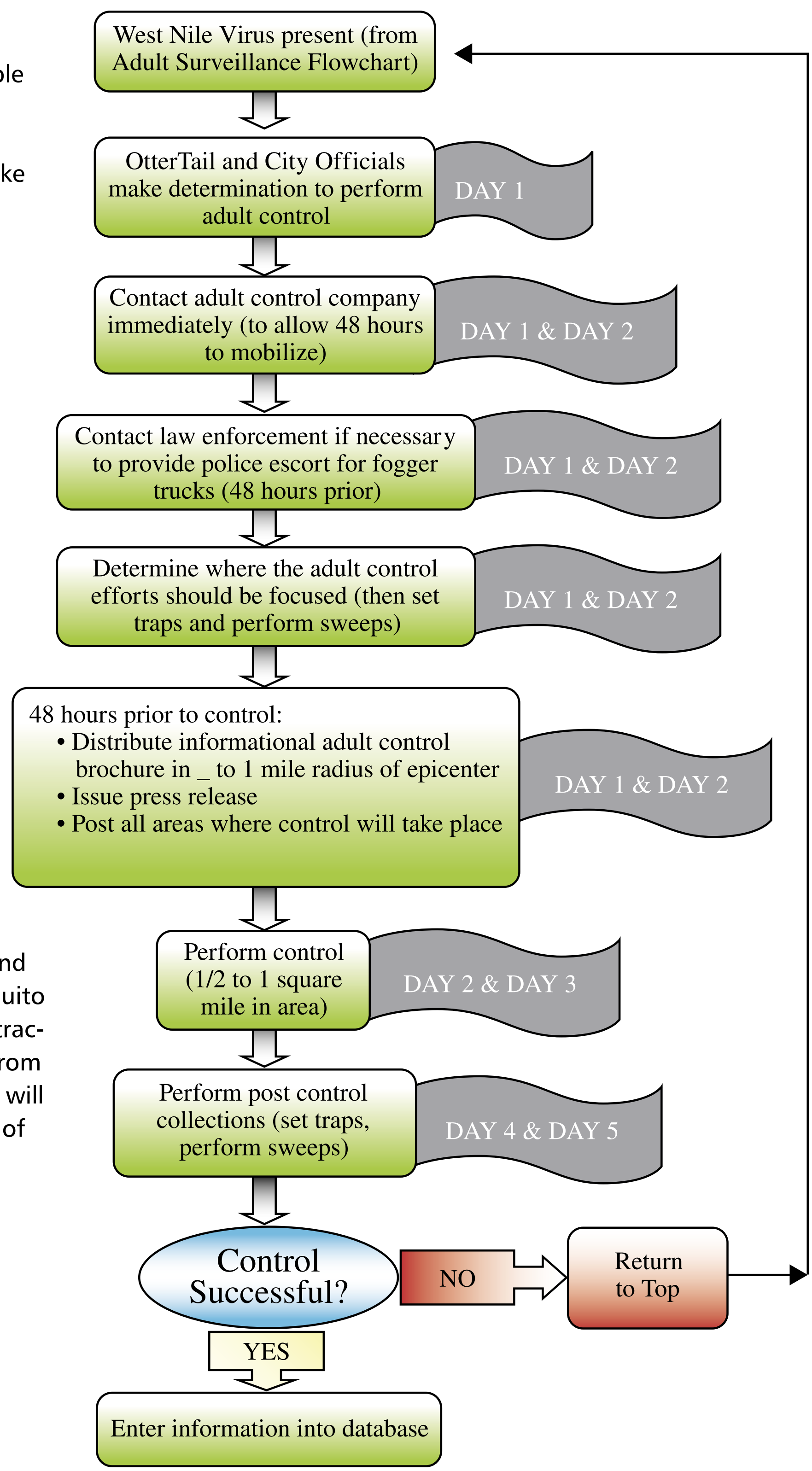


Figure 3-4 Adult Mosquito Control Flowchart

CHAPTER 4: COMMUNICATION PLAN

In 2004 a WNV communications plan was drafted in order to ensure accurate, timely and consistent communication to the public regarding the potential of WNV, personal protection from the virus, and the city's plan to test for and treat WNV-carrying mosquitoes. As a joint effort between the city of Boulder's Offices of Public Affairs and Environmental Affairs, the plan is implemented in the spring and continues until the end of the mosquito season by October. Funding is managed by the city manager's Office of Environmental Affairs.

Communication Guides

Topics and departments that will guide communication include mosquito larvae identification, Boulder County Public Health efforts, community input, city council/city manager direction, and prevention efforts. There are privacy concerns with regards to those members of the public who may contract the disease.

Seasonal Guidelines

Early/late spring: Staff/consultants hired, prevention and protection messages formulated and information outlets identified

Early Summer: Prevention and protection messages launched; early results of monitoring and treatment data communicated; communication on potential for adult mosquito spraying

Mid-Summer: Reinforcement of prevention messages; monitoring and treatment data continues to be communicated; notification of adult mosquito spraying (if necessary)

Late-Summer/early fall: estimate timeframe for end of mosquito season (depending on WNV presence and abundance during the season); monitoring and control activities slow or stop; data compilation begins

Key Messages for Success

Success of the WNV plan would mean few to no human cases of WNV, high vector larvae termination rate, and the avoidance of spraying adult mosquitoes due to the public's commitment to self-protection and habitat control. In order to reach these goals, the communication plan highlights the following four key messages to be used in all education and outreach materials:

1) self protection is the most effective way to prevent contracting WNV;

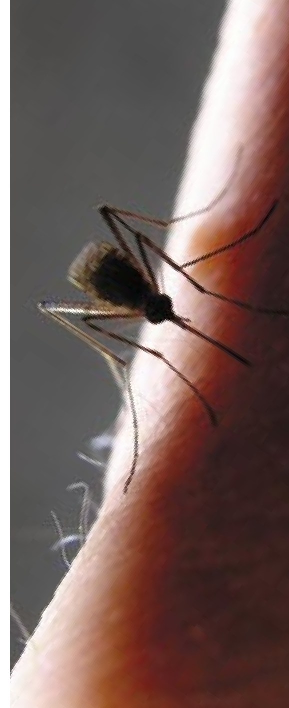
2) avoid WNV by avoiding mosquito bites;

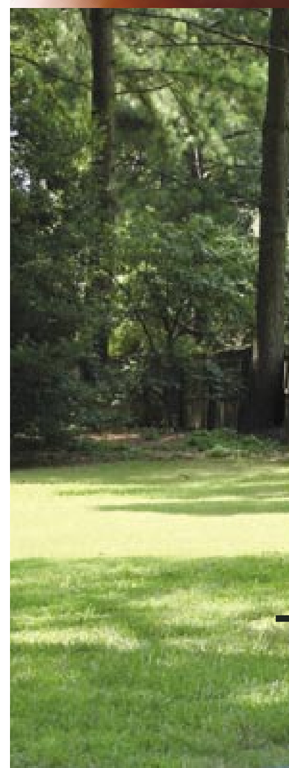
3) clean up sources of standing, stagnant water to eliminate mosquito habitats; and

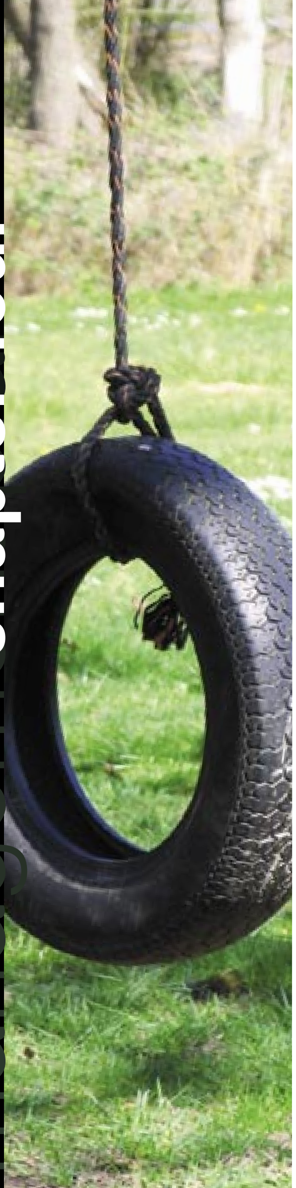
4) the public still has a responsibility to take personal precautions even if the city sprays for adult mosquitoes.

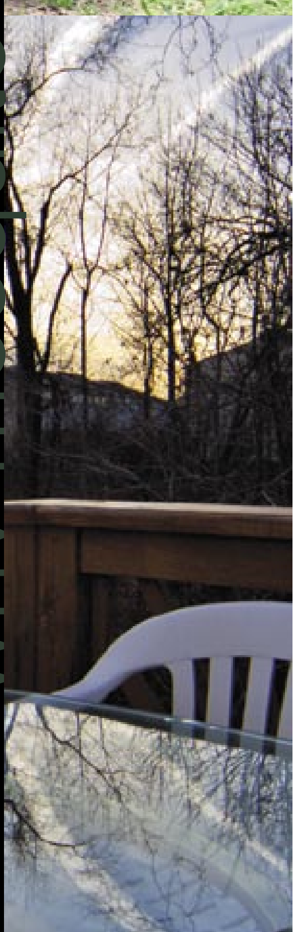
To deliver these key messages, various methods have been employed to target specific audiences.

1. **One identity/graphic that is used throughout materials** – Example: The One Bite campaign, produced by Boulder County Public Health, provides the city of Boulder with posters and flyers for distribution to the general public. The campaign materials contained information about personal protection using the "4 D's" (DEET, Dawn and Dusk, Dress, and Drain) and testimonials from persons infected with WNV in the past. Posters are displayed throughout the city of Boulder, at Open Space and Mountain Parks trailheads, Parks and Recreation facilities, libraries, senior centers and the Municipal Building to raise awareness about WNV in the Boulder community.









2. Public meetings/open houses

3. WNV hotline (303-441-4004) – Example: The WNV hotline, started in 2004, continues to be a resource for city of Boulder residents. Callers are given information from a menu service and can leave a message for city staff. Most calls received are regarding addition or removal from the No Spray List or to report potential sources of mosquitoes.

4. Videotaped presentations; use on Web and for check-out

5. Web site with details, updates, and announcements. Example: The city of Boulder's WNV website, www.ci.boulder.co.us/environmentalaffairs/ipm/2005_WNVs.html,

contains sections for current WNV activity, frequently asked questions, the city's mosquito control plan, program maps, a property checklist, and links to email the city with WNV questions and to join the mosquito spraying email list.

6. Advertising and public displays (as needed, especially for spraying)

Example: Farmer's market presence at least once/week from May through August

7. Media Relations – Example: periodic news releases about WNV activity and recommendations for preventing exposure to mosquitoes.

8. Channel 8 – update Boulder (airing starting in June)

9. Utility bill inserts – (April or May and possibly July) with extra printed for outreach distribution

City WNV Communication Team

- Neighborhood and Public Relations Coordinator
- Media Relations Coordinator
- Council Communications Coordinator
- OEA Outreach Specialist
- Integrated Pest Management Coordinator

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This West Nile Virus Mosquito Management Plan is comprised mostly of information presented in the following reports and plans:
"City of Boulder West Nile Virus Mosquito Control Plan." OtterTail Environmental, Inc. Colorado, July 2003.
"City of Boulder West Nile Virus Surveillance and Control Report, 2003 Season."
OtterTail Environmental, Inc. Colorado, March 2004.
"OtterTail Mosquito Control Field Manual." OtterTail Environmental, Inc. Colorado, 2004.

APPENDIX A: CITY OF BOULDER INTEGRATED PEST MANAGEMENT POLICY

City of boulder integrated pest management policy can be found at:
www.ci.boulder.co.us/environmentalaffairs/ipm/IPM%20policy%20rev%202002%20final.pdf

APPENDIX B: LARVACIDE AND PESTICIDE LABELS AND INFORMATION

This appendix can be found at:
www.ci.boulder.co.us/environmentalaffairs/documents/appendixb.pdf

APPENDIX C: CONTRACTS AND PERMITS

Contracted Services with City of Boulder

The initial contract is executed and put into force for 1 year. The Contract is then eligible for annual renewals for 4 additional years. These term limitations are due in part to Tabor and are not alterable. A 'scope of work' or 'schedule of work' that is redefined from year to year is necessary for annual contract renewals. Renewals are handled by the requesting department's contact in the Purchasing Department.

Putting together an initial contract is a cooperative effort between the issuing department, the City Attorney's Office and Purchasing. Purchasing facilitates the bid process, execution of the contract and renewals of the contract. The City Attorney's Office provides the Contract Boiler and assists with language additions, etc. The issuing department administers the fulfillment of the contract terms with the contractor.

A copy of the current contract and scope of work can be found at:
www.ci.boulder.co.us/environmentalaffairs/documents/appendixc.pdf

Permits

A wetland permit is required for larval mosquito control using a pesticide in all regulated city of Boulder wetlands, as stated in the 1981 Boulder Revised Code, Title 9 Land Use Regulation, Chapter 12 Wetlands Protection (and amended by Ordinance No. 7338).

The following is a link to the wetland ordinance. Section 9-12-12 covers the renewal requirements. www.ci.boulder.co.us/cao/brc/title9.html

A copy of the current contract and scope of work can be found at:
www.ci.boulder.co.us/environmentalaffairs/documents/appendixc.pdf

APPENDIX D: MOSQUITO-BORNE DISEASES THAT MAY OCCUR IN COLORADO

There are currently four arboviruses found in Colorado that are transmitted by mosquitoes: WNV, Western Equine Encephalitis, St. Louis Encephalitis, and California Encephalitis. Arbovirus, which is short for arthropod-borne virus, is a group of viruses that are spread mainly by blood-sucking insects, such as mosquitoes. Encephalitis is an acute inflammation of the brain tissue. It can be caused by a variety of agents, but viruses are the most common cause. The following chapter describes these viruses.

Western Equine Encephalitis

Western Equine Encephalitis (WEE) is found throughout North, Central, and South America. However, the majority of the cases in North America have been reported from the rural western United States. WEE is spread mainly by the vector mosquito C. tarsalis. Birds are the primary hosts for WEE because of their ability to act as reservoirs to the virus and allow it to replicate easily (Leighton 2000). Birds are infected in the spring by virus-carrying mosquitoes; the virus replicates and amplifies in the birds, other mosquitoes feed on the birds, the newly infected mosquitoes can infect more birds, and the cycle continues. WEE is common in the summer, with peaks in July and August when populations of the vector mosquito are the highest (Nadalur and Urban 2002). The risk of disease in humans and horses varies from year to year depending on the number of infected mosquitoes. A vaccine is available for horses, but there is no vaccine for general use in humans because of the small amount of verified WEE cases.

Saint Louis Encephalitis

The St. Louis Encephalitis (SLE) virus was first recognized in 1933 in St. Louis, Missouri. Currently, the virus occurs throughout the United States, with higher concentrations in the Mid-West and Southern states with small (fewer than 30 people affected), isolated regional outbreaks (CDC 2001). Birds are immune hosts of the virus, which is transmitted between birds and from birds to humans and other animals by the blood-sucking mosquitoes (CDC 2001). The virus is transmitted and spread primarily by mosquitoes of the genus Culex. As with WEE, humans are a dead-end host for SLE. Most humans that are infected never show any symptoms of the disease. Currently, there is no vaccine against SLE.

California Encephalitis

The California Encephalitis (CE) virus was first named after a human case diagnosed in 1946 in Kern County, California (Soliman, No date). Since that time, most cases have been linked with the La Crosse virus. The La Crosse virus occurs in north-central states, primarily in the upper Mississippi River Valley, and is transmitted by the treehole mosquito, Aedes triseriatus. The vertebrate hosts of the virus are primarily small woodland mammals, such as squirrels and chipmunks, which harbor the virus until it is passed to other mosquitoes. The virus is able to survive the winter within the vector mosquito (CDC 2001). Only about 70 cases of La Crosse encephalitis are reported annually.

West Nile Virus

West Nile Virus (WNV) was first found in the United States in 1999 in New York City and has since spread to 43 states (U.S. Geological Survey [USGS] 2002). The virus is carried long distances by infected birds and then spread locally by mosquitoes that bite the infected birds. The virus is primarily transmitted by mosquitoes of the genus Culex. In Colorado, the mosquito species Culex tarsalis and Culex pipiens are known to be involved in the transmission cycle of the virus. C. tarsalis is considered more likely to transmit the virus to mammals, such as horses and humans, which are dead-end hosts. Humans and horses are described as dead-end hosts for the virus because, once it has been contracted, they cannot transmit the disease.

The highest risk of WNV infection for humans is usually August through early September.

Because the status of WNV in Colorado is changing rapidly, the most current information on WNV in the state can be found by referring to the WNV educational campaign web page at: http:// www.fightthebitecolorado.com. For additional information on WNV, refer to the Centers for Disease Control and Prevention (CDC) web page at: www.cdc.gov/ncidod/dvbid/westnile/index.htm or by calling the Colorado Health Education Line for the Public at 1-877-462-2911. The statewide, toll-free hotline, which is staffed by trained operators, is open from 7 a.m. to 11 p.m. seven days a week. Callers to the hotline will be able to obtain information in English and Spanish on a wide variety of topics relating to WNV, including the following:

- Information on repellants and how they should be used both on adults and children.
- Symptoms of WNV, however, hotline operators will not be able to help diagnose whether a person has contracted WNV. Rather such individuals will be referred to their personal physician for diagnosis and treatment.
- Methods of preventing WNV.
- How pets might be affected by WNV.

West Nile Virus Pathway

WNV is carried long distances by infected birds on seasonal migration patterns and then spread locally by mosquitoes that bite the infected birds. The virus is primarily transmitted by mosquitoes of the genus Culex. In the Boulder Area, the primary species of Culex are Culex tarsalis and Culex pipiens. These two species are considered the most likely to transmit the virus to mammals, such as horses and humans, which are dead-end hosts. Humans and horses are described as dead-end hosts for the virus because; once it has been contracted, they cannot transmit the disease due to inadequate amounts of viremia, or virus circulation in the bloodstream to infect other mammals or mosquitoes. Mosquito of the Culex genus overwinter and; therefore, can continue the disease the following spring.

West Nile Virus in the U.S.

WNV was first isolated in 1937 and known to cause asymptomatic infection and fevers in humans in Africa, West Asia and the Middle East. First detected within the U.S. in the state of New York in 1999, WNV has spread from East to West across the United States by mosquito hosts and carrier birds. During 1999 and 2000, WNV encephalitis was found in U.S. residents in three northeastern states with a total of 83 human cases and 9 reported deaths. WNV spread westward in 2001 occurring in 10 states having 66 cases and 9 deaths. As of 2005 WNV has been detected in all states except for Alaska and Hawaii. There is currently no WNV vaccine for humans, but there is for horses.

West Nile Virus in Colorado

In 2002, Colorado had its first cases of humans infected by WNV with 14 documented human cases and no reported WNV-related deaths (CDPHE 2004). These 14 cases occurred despite a severe drought. Additionally, 138 bird, 3 sentinel chicken flocks, and 380 horses tested positive for WNV in 2002 (CDPHE 2004). During the 2003 mosquito season, Colorado lead the nation in WNV human cases (2,945) and WNV-related deaths (55) (CDPHE 2004). This is likely due primarily to Colorado's wet spring and hot summer increasing mosquito breeding populations and earlier emergence as described earlier. The onset of human symptoms was first documented with less than 10 cases on June 30, 2003, by CDPHE. The number of cases from June to September followed a Bell shaped curve with the major peak in August that year.

West Nile Virus in Boulder County

By the end of 2003 there were 430 WNV infections reported to Boulder County Public Health, 378 were diagnosed with WNV Fever and 52 with either WNV Encephalitis or Meningitis. Based on monitoring data among human cases from 2003, it is estimated that there were approximately 7,800 individuals infected with WNV in Boulder County (only 430 reported illnesses) (Harmon 2004).

APPENDIX E: GENERAL OVERVIEW OF MOSQUITO HABITAT AND BIOLOGY

Larval Habitat

Mosquitoes successfully inhabit almost every kind of collection of water. A "breeding site" can be any place that will hold water for a week or more after rainfall. Prime breeding sites include marsh edges, short-grass ditches, tire ruts, hoof prints, discarded tires left outdoors, poorly maintained bird baths, holes in trees, clogged rain gutters, unused swimming and plastic wading pools, and pots and pans with standing water, and many other habitats that will hold stagnant water (New York City Department of Health and Mental Hygiene 2003, Metropolitan Mosquito Control District [MMCD] 2002). The most prolific breeding sites in the city are probably flood-irrigated lands, and seasonally wet/dry locations when stagnant water is present.

Some areas that do not support mosquitoes include moving water (rivers, streams, and creeks), deeper lakes, ornamental ponds, and duck ponds. Other conditions that are unfavorable for breeding of mosquitoes are turbulence and the presence of natural predators.

Adult Habitat

In the daytime, adult mosquitoes avoid adverse environmental conditions, such as intense heat, by taking refuge in resting areas known as "harborage sites". Typically, these resting areas are composed of natural vegetation, including forests, tree stands, grass, shrubs, or other foliage. Ideal resting areas are generally shaded with cooler daytime temperatures and high relative humidity. These conditions are typically found in forests or tree stands that have a canopy, and dense underbrush. Wetlands also may be present nearby. Other resting sites include culverts, hollow logs, areas underneath decks, shaded sides of buildings, basements, and garages.

General Mosquito Biology

Mosquitoes develop through four general stages in their life cycle. The following sections describe these four stages.

Eggs – All mosquitoes must develop in water before they can fly. The adult female mosquito, after taking a blood meal, will search for a place to lay her eggs. Culex mosquitoes lay eggs in clusters, also called egg rafts, on the water's surface. C. tarsalis lay eggs in rafts on the surface of permanent and semi-permanent clear ground pools, springs, and ditches. In late summer, they also lay eggs in temporary pools and containers that contain standing water. C. pipiens use standing or slow-moving water that contains decaying organic materials to lay their eggs.

Larvae – Larvae develop in shallow water. They have four growth stages known as instars (Figure D-1). They are found in the water hanging head down just below the surface because the larvae breathe through a respiratory siphon at the tail end of their body that breaks the surface of the water. When larvae first hatch, they are less than _ inch long and they grow to be approximately _ inch long by the fourth instar.

The larvae of C. tarsalis and C. pipiens are found in somewhat different habitats. C. tarsalis larvae are found in a wide variety of semi-permanent and permanent sources of water in both rural and urban areas (Nielsen et al. 2002). They occupy a wide variety of either fresh or polluted water habitats, usually in open, sunlit locations (Harmston and Lawson 1963). In contrast, C. pipiens larvae are found in a wide variety of natural and artificial sources of water that often are highly polluted with organic wastes (Nielson et al. 2002, Harmston and Lawson 1963). They have been found in containers of various types, catch basins, ornamental pools, cesspools, swimming pools that are not completely drained, ditches, and tree holes (Nielsen et al. 2002).

Pupae – At the end of the fourth instar, the larva molts into a pupa. The pupa is a cocoon-like stage when the adult mosquito is forming. This stage typically lasts about 2 days; however, the amount of time spent in the pupa may vary depending on water's temperature. The mosquito does not feed during the pupa stage, but when disturbed, will tumble as it avoids danger.

Adult –When the adult is fully formed, it breaks through and emerges from the pupal skin. It rests for a short time on the water surface while its wings expand and dry. Male mosquitoes usually emerge first and form a swarm where they will mate with females as they emerge from their pupae. Females mate only once

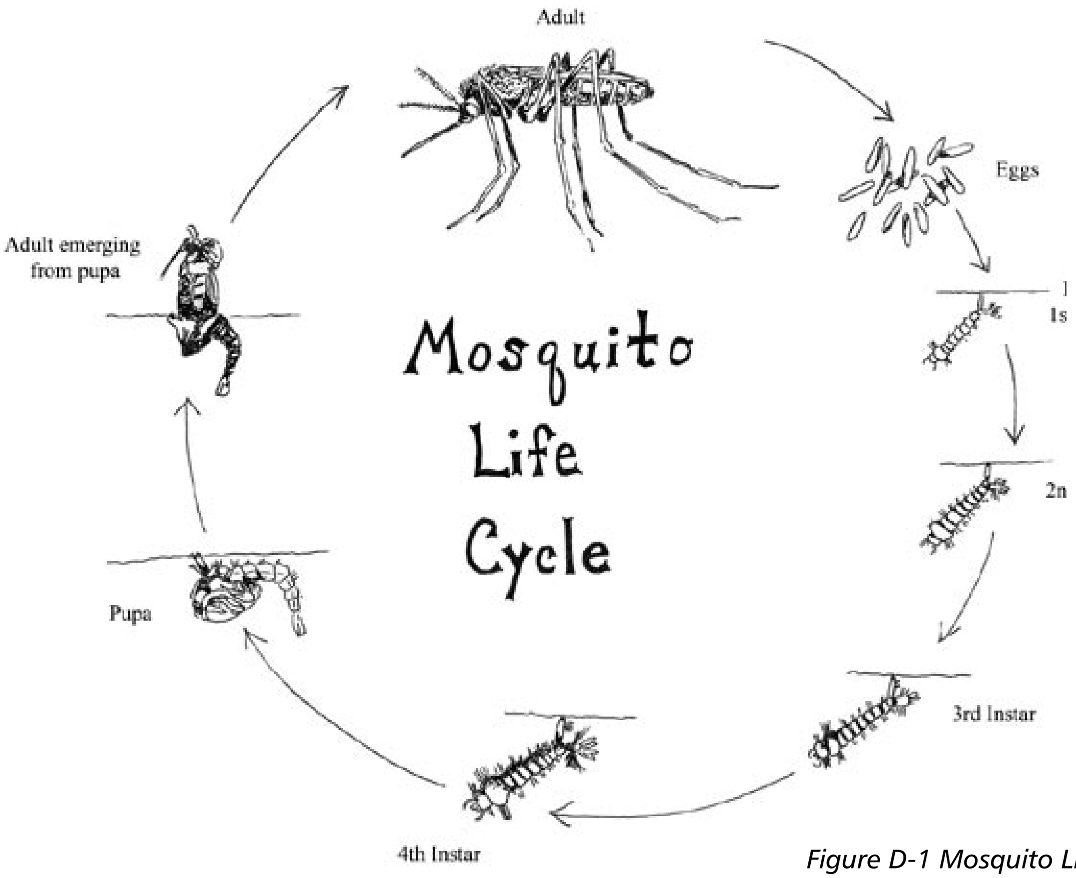

Figure D-1 Mosquito Life Cycle

and store sperm in their bodies to fertilize their eggs as they are laid. Once the female has mated, she flies off in search of a blood meal to obtain the proteins necessary for laying eggs. Males and females feed on plant nectar for energy.

A number of factors influence the blood feeding of the adult female. They include humidity, wind, temperature, light, and animal emanations (such as respiration or body heat). For most mosquitoes, the primary period for feeding on blood is between sunset and midnight during the summer. A second feeding period occurs around sunrise. This feeding behavior may change during the spring and fall, when daytime conditions favor mosquito activity over evening conditions. Temperatures above 55 degrees F and humidity levels at or in excess of 70 percent are optimum feeding conditions.

Mosquitoes of the genus Culex can overwinter as gravid (egg bearing) females. This characteristic results in populations that are low in numbers in the spring but grow geometrically during August and September. Because the populations of mosquitoes increase greatly late in the summer, potential vectors and disease transmission are most prevalent at this time.

C. tarsalis breeds several generations per year. Females overwinter in protected places, including caves, abandoned mines, and cellars (Harmston and Lawson 1963). Adults prefer to feed on birds, but will bite humans and other mammals (Nielson et al. 2002; Harmston and Lawson 1963). Feeding occurs near dusk and after dark (Nielsen et al. 2002). Its life cycle varies from 4 days to 30 days, depending on conditions. C. tarsalis commonly travels up to 2 miles for a blood meal. Collections have been made at elevations up to 10,000 feet (Harmston and Lawson 1963).

C. pipiens females hibernate in cellars, basements, and other protected sites (Harmston and Lawson 1963). Studies suggest that birds are the major hosts of C. pipiens because it takes blood meals from them more than 95 percent of the time. Mammals constitute the rest, with humans representing less than 1 percent of the total (Nielsen et al. 2002).

C. tarsalis is probably the main carrier of WNV because of its affinity to take blood meals from birds. At least 120 bird species and eight mammal species have been infected (USGS 2002). Corvids (crows, magpies, ravens, and jays) seem to be affected more than other species; however, because many corvids die when infected, they are not an ideal host for the virus. Other species, such as house sparrows, do not seem to die as readily when infected and are therefore a more effective host for the virus.

APPENDIX F: CITY-WIDE STAFF WEST NILE VIRUS TASK FORCE

The city has set up "teams" of staff to help in the decision-making and implementation of the West Nile Virus Mosquito Management Plan.

Technical Team

The technical team is a group of city staff members who have knowledge and expertise in pest control and, as best known to date, West Nile Virus. This group consists of:

- Team Lead, WNV Project Co-Manager, Office of Environmental Affairs (OEA)
- OEA intern
- Open Space and Mountain Parks
- Parks and Recreation
- Transportation & Utilities Maintenance
- Monitoring and Control Consultant

Public Affairs Team

The public affairs team is a group of public information specialists from throughout the city that are led by Public Affair's staff. The group is tasked with developing and implementing the city's communication plan on WNV to both internal and external customers of the city.

- Team Lead - WNV Project Co-Manager, Public Affairs
- Public Affairs
- City Manager's Office
- OEA
- PIT (as needed) -
  Public Information Team (public information representatives from departments city-wide)

City-wide Staff Task Force

The city-wide task force is made up of representatives of the departments and offices within the city. The task force shares information, asks questions of the technical and public affairs teams, coordinates individual department efforts with other departments, and brainstorms ways to engage city staff and the public in the prevention of WNV.

Task Force Co-Leaders:

- WNV Project Co-Manager, Public Affairs
- WNV Project Co-Manager, OEA

Participating Departments

- City Manager's Office
- Environmental Affairs
- Fire Department
- Housing and Human Services/Senior Services
- Human Resources
- Library
- Municipal Court
- Open Space and Mountain Parks:
- Parks and Recreation
- Public Affairs
- Public Works

2006 WNV Mosquito Management Plan

Prepared by: City of Boulder

Office of Environmental Affairs

In collaboration with

OtterTail Environmental Inc.